1985

Testicular Volume And Its Correlation With The Cytogenetics Of Mental Retardation

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Testicular Volume and Its Correlation with the Cytogenetics of Mental Retardation

by
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Submitted in partial fulfillment
of the requirements for the degree of
Doctor of Philosophy

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May 1985

ABSTRACT

Abnormal testicular volume can be associated with various mental retardation (MR) syndromes. For example, bilateral testicular enlargement with no evidence of any endocrinological disturbance has been described in some families with non-specific X-linked MR, both fragile X positive and negative. In contrast, a fragile X negative kindred with non-specific X-linked MR in which affected males had small to normal size testes has also been reported. Microorchidism has, in addition, been associated with various chromosome abnormalities such as the Klinefelter syndrome, reciprocal translocations, and autosomal abnormalities. Based on these reported observations, the present study was initiated to determine the usefulness of abnormal testicular volume as a clinical marker in the cytogenetic evaluation of MR syndromes.

The testes of institutionalized, non-Down syndrome males were measured with sliding calipers for the determination of testicular volume (V). Macro-orchidism ($V \geq 25$ ml) was found in 20.8% of residents, micro-orchidism ($V \leq 15$ ml) in 43.1%, normal testicular volume ($15 < V < 25$) in 33.3%, and cryptorchidism in 2.8%.

Cytogenetic investigations revealed an overall frequency of 3.1% for the fragile X syndrome among institutionalized (non-Down syndrome) males. A significantly higher proportion of macro-orchid males (30.3%) had the

fragile X as compared to males with either micro-orchidism (1.2%) or normal testicular volume (1.2%).

Examination of the medical records of fragile X negative probands showed that a further 10.4% of institutionalized (non-Down syndrome) males had a family history of MR which was suggestive of fragile X negative non-specific X-linked MR. There was no significant difference in the distribution of fragile X negative non-specific X-linked MR in males with macro-orchidism (10.9%), micro-orchidism (9.7%) or normal testicular volume (9.1%). The preferential association of macro-orchidism with the fragile X syndrome clearly establishes the use of enlarged testicular volume as a clinical marker in the diagnosis of this MR syndrome.

Chromosome abnormalities other than the fragile X were found in 3.6% of institutionalized (non-Down syndrome) males. Although a large number of the chromosome anomalies detected in this study were found in micro-orchid males, there was no significant difference in the distribution of non-fragile X chromosome abnormalities between males with micro-orchidism (4.2%), macro-orchidism (2.4%), and normal testicular volume (3.0%). A significantly greater number of micro-orchid males (39.8%) were found to have spastic/paralytic disorders as compared to males with either macro-orchidism (4.2%) or normal testicular volume (15.6%). The above findings show that micro-orchidism is not a useful clinical marker in the evaluation of MR males with possible

chromosome abnormalities. The reduced testicular size may, however, be indicative of MR resulting from neurological damage.

The karyotypic abnormalities detected in this survey included: 46,XY,del(5)(p14→pter); 47,XY+inv dup(15)(p13→q12); 47,XXY; 47,XYY; 46,XY,del(10)(p13→pter); 46,XY,dup(21)(q22); 46,XY,der(8q+)?; 45,XY,-13,-14, +t(13q14q); 46,XY,t(14;15)(q32;q13); 46,XY,t(5;14)(q22;q31); 46,XY,t(16;22)(p12;q13); and 46,XY,t(7,16)(q11;p13). An interesting finding was the occurrence of the supernumary inv dup(15) chromosome [47,XY,+inv dup(15)(p13→q12)] among institutionalized males at a frequency approximately equal to the cri-du-chat syndrome [46,XY,del(5)(p14→pter)].

Dedication

To the families of all of our patients.

ACKNOWLEDGEMENTS

The author wishes to thank the following people:

Dr. A. H. Martin for allowing me the privilege to do graduate work in the Department of Anatomy.

Dr. F. R. Sergovich for his patience, supervision, and friendship.

Dr. B. Goldberg, Director of Treatment, Training and Research, and Mr. Eugene Sorin, Facility Administrator at the Children's Psychiatric Research Institute (CPRI), London, for allowing me access to the facilities and resources of the Institute. I would also like to express my appreciation to the various other institutes of the Ministry of Community and Social Services which participated in this study: Huronia Regional Centre, Orillia, Ontario; Southwestern Regional Centre, Blenheim, Ontario; Oxford Regional Centre, Woodstock, Ontario, and St. Thomas Adult Rehabilitation and Training Centre, St. Thomas, Ontario. While many individuals were of great assistance, Dr. E. Crawford of Huronia should be especially recognized for her help in facilitating the study and many hours involvement in family follow-up and counselling.

Dr. J. Pozsonyi and Ms. G. Sheridan for performing the testicular measurements and collection of clinical data.

Ms. H. Cheung for help with the statistical analyses.

Mrs. P. Allgrove for her care and efficiency in typing not only the initial manuscript, but also all reports, letters and papers.

Mrs. P. Noulty (Accu Type Word Processing Centre) for an excellent final manuscript.

Ms. R. Cooper-Dickout for her expert help in the photographic work.

I wish to convey my sincere appreciation to Peggy LeBlanc, Julie Needham, Anne Seddall, Donna Wilker and Rosemary Windmoller, not only for all the help they have given me, but also for their constant and warm friendship, encouragement, and moral support throughout this endeavour.

Most especially, I would like to thank Pat, Ken, Jason, Janice, and Justin Rodney for opening their home and their hearts to me.

I would also like to acknowledge the friendship and humour of the late Dr. J. Pozsonyi who died before the completion of this work.

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BrdU	5-bromodeoxyuridine
CNS	central nervous system
CPRI	Children's Psychiatric Research Institute
DAPI	4', 6-diamidino-2-phenyl-indole
DHF	dihydrofolate
DNA	deoxyribonucleic acid
dUMP	deoxyuridine monophosphate
dTMP	deoxythymidine monophosphate
fra(X)	fragile X
FdU	5-fluorodeoxyuridine
G6P	glucose-6-phosphate dehydrogenase
IQ	Intelligence quotient
inv(3c)	pericentric inversion of the centromeric region of chromosome 3
l	length
MCA	multiple congenital anomalies
MCH	mean corpuscular hemoglobin
MG	methyl green
MR	mental retardation
PKU	phenylketonuria
p_i	success rate
$\hat{p}_i$	success probability
P_o	stratified overall frequency
NOR	nucleolus organizer region
RLFP	restriction length fragment polymorphism
SOD	superoxide dismutase
THF	tetrahydrofolate
V	volume
w	width
XLMR	X-linked mental retardation

I Introduction

Individuals with chromosome abnormalities are usually characterized by generalized growth and psychomotor retardation (Vogel and Motulsky, 1979). One of the features commonly reported in karyotypically abnormal males is hypogonadism. For example, a symptom consistently observed in the Klinefelter syndrome (47,XXY) is very small testes (approximately 1/3 normal size). This reduced size is due to abnormal number and structure of the seminiferous tubules which results in azoospermia and loss of volume. Severe hypogonadism and cryptorchidism have also been found in males with apparently balanced, and usually de novo, translocations (Chandley et al, 1976; Handelsman and Smith, 1983) and appear to be quite common in autosomal trisomy and deletion syndromes (Summitt, 1979).

On the other hand, macro-orchidism had been found to be a common but not consistent feature in males with non-specific X-linked mental retardation (XLMR) (Turner et al, 1975). In 1978, Turner et al reported that males with the fragile X [fra(X)] positive form of non-specific XLMR were macro-orchid whereas XLMR males who were fra(X) negative had normal testicular volume. These observations were complicated by subsequent reports of fra(X) positive males with normal testicular volume, and of macro-orchid males who were fra(X) negative (Sutherland and Ashforth, 1979; Jennings et al, 1980; Herbst et al, 1981; Fishburn et

al, 1983). In addition, Fox et al, (1980) reported a family with fra(X) negative non-specific XLMR (Renpenning et al, 1962) in which affected males had small to normal size testes. It was thus unclear to what degree non-specific XLMR was genetically heterogeneous and whether testicular volume could be a useful anatomical marker in delineating cytogenetic disorders.

This study was initiated to determine the degree of nonrandomness in the occurrence of abnormal testicular volume in various cytogenetic MR syndromes. Specifically, the goals of this investigation were:

(1) to determine the proportions of institutionalized males who have macro-orchidism, micro-orchidism, and normal testicular volume [Down syndrome patients were excluded because it is well recognized that hypogonadism was often associated with this specific disorder (Summitt, 1979)].

(2) to identify and classify patients with chromosome abnormalities, including the fra(X), in each volumetric group. Cytogenetic analysis was performed on male residents on the basis of testicular volume regardless of a family history of MR. This is in contrast to previous reports in which the selection criterion for the fra(X) syndrome generally was based on a positive history of MR.

(3) to determine whether any correlation is present between

(a) micro-orchidism and specific autosomal chromosome syndromes as well as non-specific XLMR;

(b) macro-orchidism and non-specific XLMR with special reference to the fra(X) syndrome; and

(4) to determine whether the correlations in (3) may have usefulness in the diagnostic assessment of mentally retarded individuals.

II Literature Review

The presence of an additional chromosome 21 in Down syndrome was first described by Lejeune et al in 1959. Previous to the finding of this chromosome abnormality, Down syndrome was a well defined clinical entity characterized by mental retardation, growth failure, and a variety of physical anomalies. The finding of trisomy 21 in Down syndrome led to extensive cytogenetic studies of mentally retarded and congenitally malformed individuals with the expectation that other chromosome anomalies would also be associated with various phenotypic abnormalities. Cytogenetic surveys were also extended to consecutive newborns and aborted fetuses in an attempt to establish the overall frequencies of chromosomal abnormalities.

Systematic chromosome analyses on patients with multiple deformities and/or MR did reveal many other specific chromosomal aneuploidies. While some of these chromosomal syndromes could be defined clearly by clinical features, an accurate phenotype-karyotype correlation was not always possible due to the lack of distinctive clinical features in many cases (Lubs and Ruddle, 1970; Vogel and Motulsky, 1979). Phenotypic variability was described even among patients with identical chromosomal abnormalities (Lubs and Ruddle, 1970).

A common feature of many chromosomal disorders is the presence of minor congenital anomalies. The clinical recognition of any such associated physical feature would be

of primary importance in the identification of individuals with possible karyotypic abnormalities.

A. Chromosome Anomalies in Neonates and Children

Cytogenetic abnormality is a significant cause of birth defects and fetal loss. The incidence of chromosomal abnormalities in liveborn infants has been estimated to be 0.6% (Nielsen and Sillesen, 1975). The overall incidence of these abnormalities is even higher if one considers all conceptuses. It has been postulated that 7.5% of all conceptuses carry a chromosomal abnormality, and that approximately 50% of spontaneous abortions exhibit abnormal karyotypes (Carr, 1971). The incidence of chromosome abnormalities in those who are liveborn has been estimated as follows (Nielsen and Sillesen, 1975): 0.22% have gonosomal aneuploidy (0.16% male and 0.06% female), 0.15% have autosomal aneuploidy (with Down syndrome being the most prevalent: 0.10%), 0.19% possess balanced structural rearrangements (0.17% balanced translocations and 0.02% inversions), and 0.05% have unbalanced structural rearrangements. Approximately 80% of balanced structural rearrangements found in neonates are familial in origin (Evans, 1977). Carriers of balanced structural chromosome defects, although phenotypically normal, have approximately 15% less liveborn offspring than their karyotypically normal siblings (Jacobs et al, 1970).

The overall incidence of chromosome anomalies in older children was 0.48% (Patil et al, 1977) as compared to 0.6% found in neonates (Nielsen and Sillesen, 1975). Sex chromosome abnormalities and balanced autosomal structural rearrangements in 7-8 year old children were found in the same frequency as in newborn surveys. A lower incidence of autosomal anomalies (0.04%) was found in the older children (Patil et al, 1977) as compared to neonates (0.14%) (Nielsen and Sillesen, 1975). This lower percentage is probably due to the death or institutionalization of affected children before age 7-8 years (Patil et al, 1977). For example, about 40% of Down syndrome patients die by their first year (Gustavson et al, 1977).

B. Cytogenetics and Mental Retardation

1. Cytogenetic Surveys of Mentally Retarded Individuals

Several cytogenetic investigations of mentally subnormal persons have been performed (Tables 1-3). The majority of surveys have dealt with institutionalized individuals, either as unselected (Table 2) or selected groups based on phenotypic abnormalities (Table 3). Only two surveys of a complete population of mentally retarded individuals, residing at home or in institutions, have been presented (Speed et al, 1976; Rasmussen et al, 1982) (Table 1). The results of these latter two studies will be reviewed first.

(a) Complete Populations

The first cytogenetic survey of an entire population of the mentally subnormal, both institutionalized and residing at home, was an unbanded chromosome study (using solid staining) from northeast Scotland (Speed et al, 1976). Of the 2,770 individuals examined, 297 (10.7%) were detected to have chromosome abnormalities. Individuals with Down syndrome accounted for 250 (9%). Sex chromosome anomalies were found in 25 (0.9%) males and 6 (0.5%) females. Deletions and supernumaries comprised the remaining 0.3% of autosomal anomalies. No balanced structural rearrangements were found.

The second survey was performed on a registered population of mentally retarded persons, both institutionalized and residing at home, in a limited area of Denmark (Rasmussen et al, 1982). The karyotypes of 1,905 individuals were examined by chromosome banding techniques. Of the 359 (18.8%) individuals who were detected to have a chromosome anomaly, 27(1.4%) had Down syndrome, 39(2.1%) had an autosomal anomaly other than Down syndrome, 33 (0.7% male and 1.0% female) had a sex chromosome anomaly, and 18 (0.9%) had a balanced re-arrangement.

The incidence of individuals with chromosome anomalies among the total number of mentally retarded individuals examined was higher in the Danish survey (18.8%) (Rasmussen et al, 1982) than in the Scottish survey (10.7%) (Speed et

al, 1976) (Table 1). However, the incidence of MR in the Scottish area (0.63%) (Speed et al, 1976) was almost twice that in the Danish area (0.39%) (Rasmussen et al, 1982) (Table 1). This difference in frequency of reported cases of MR can be based on the total number of registered mentally retarded persons, which is determined by the type of population studied (urban or rural) as well as socio-economical factors such as the availability of medical consultation, economic aid, and treatment (Rasmussen et al, 1982). When the frequency of individuals with chromosome abnormalities in the total population was compared between the two areas, the incidence was approximately the same in both studies: 0.067% in Scotland (Speed et al, 1976) and 0.064% in Denmark (Rasmussen et al, 1982) (Table 1). This finding is in agreement with the results of neonatal surveys (Jacobs et al, 1974; Nielsen and Sillesen, 1975), in which no differences were reported in the incidence of chromosomal abnormalities between the Scottish and Danish areas.

The incidences of Down syndrome, supernumary chromosomes, and sex chromosome anomalies (as a percentage of the total population) were found to be similar in the two studies (Speed et al, 1976; Rasmussen et al, 1982) (Table 1). However, the incidences of structural aberrations and balanced rearrangements were higher in the Danish study (Rasmussen et al, 1982) (Table 1). This higher incidence could be due to the fact that the survey of Rasmussen et al

(1982) used banding techniques, in which a higher number of small aberrations and rearrangements, easily missed with solid staining, could be detected (Breg, 1977; Nielsen et al, 1982).

(b) Institutional Surveys

(i) Unselected Groups

Several surveys have been published in which institutionalized but otherwise unselected MR patients were karyotyped. The results of these surveys are summarized in Table 2. Chromosome banding techniques were used consistently in the studies of Jacobs et al (1978), Ally and Grace (1979), Klein et al (1980), and Brondum-Nielsen et al (1983c). In other studies (Faed et al, 1979; Gripenberg et al, 1980; Kondo et al, 1980), the chromosome banding technique was applied only to examine further the chromosome abnormalities detected initially by solid staining.

The incidence of institutionalized MR patients with a chromosome abnormality was approximately 15.9% (Table 2). The most common anomaly was Down syndrome (13.2%) (Table 2). These findings are within the range of percentages reported by Speed et al (1976) and Rasmussen et al (1982) (Table 1).

Sex chromosome anomalies were found in 0.7% of patients (Table 2). The surveys of Speed et al (1976) and Rasmussen et al (1982) reported almost twice this frequency of sex chromosome abnormalities (Table 1). Individuals with sex

chromosome aneuploidy, however, are known to be concentrated among the mildly retarded (Court-Brown, 1969; Jacobs et al, 1978). Since the surveys of Speed et al (1976) and Rasmussen et al (1982) included non-institutionalized as well as institutionalized MR individuals, it is possible that a greater number of the mildly retarded living at home were karyotyped. This sampling method appeared to result in a higher incidence of reported sex chromosome anomalies (Jacobs et al, 1978).

As shown in Table 2, approximately 1.4% of institutionalized patients had unbalanced structural rearrangements or trisomies other than that of chromosome 21. This percentage lies within the values reported by Speed et al (1976) and Rasmussen et al (1982). The incidence of such autosomal anomalies in the study of Speed et al (1976) might have been underestimated because it was an unbanded survey. The incidence of unbalanced karyotypes was found to be inversely proportional to the age of the population studied: the older the sample population, the fewer the number of autosomal trisomies and unbalanced rearrangements were found (Jacobs et al, 1978). This lower incidence of unbalanced autosomal anomalies among older individuals can be explained by the increased rate of early mortality found among patients with autosomal aneuploidies (Jacobs et al, 1977; Patil et al, 1977; Gustavson et al, 1977).

Balanced chromosomal rearrangements are the only

chromosomal aberrations in which contrasting incidences were reported (Jacobs et al, 1978). The incidence of balanced structural rearrangements in the majority of institutionalized surveys was approximately 0.6% (Table 2). Rasmussen et al (1982) reported an incidence of 0.3% for balanced rearrangements whereas Speed et al (1976) found no such rearrangements. Based on a neonatal incidence of 0.2% for balanced rearrangements (Nielsen and Sillesen, 1975), Speed et al (1976) would have expected to find at least 6 rearrangements (6/2,770 = 0.2%) (Rasmussen et al, 1982). However, as the survey of Speed et al (1976) was an unbanded study, it is possible that such balanced rearrangements as well as other small autosomal aberrations were simply missed (Breg, 1977; Nielsen et al, 1982).

(ii) Selected Groups

The criteria for selection in the majority of these studies were based on severely abnormal individuals who possessed a number of physical malformations and in whom there was no clearly defined reason for MR. The results of these surveys [the Down syndrome patients from the surveys of Chen et al (1970), Corey et al (1971), Nelson and Smart (1982), Kodama (1982), and Retief et al (1983) were excluded] are summarized in Table 3.

A causal relationship between abnormal phenotypes and karyotypes was first established by the Madison blind study

(Summitt, 1969; Daly, 1970; Magnelli, 1976; Doyle, 1976). It was shown that unbalanced cytogenetic anomalies were present in phenotypically abnormal individuals and absent in normal ones. Other studies (Table 3) have since shown that approximately 8% of selected MR patients have a chromosome abnormality, as compared to approximately 3% of the unselected MR patients (excluding Down syndrome patients) (Table 2).

The incidences of balanced structural rearrangements in the surveys of unselected and selected mental retardates are 0.60% and 0.74% respectively. Both these values are higher than the incidence of 0.20% found in neonates (Nielsen and Sillesen, 1975). The high percentage of balanced structural rearrangements among the mentally retarded was first noted by Jacobs (1974). She suggested that these translocations were due to de novo mutations and that the associated MR could be caused by either: a) a loss of chromosome material in the translocation area, b) a position effect, and c) a point mutation.

2. Chromosome Heteromorphisms

An association between chromosome heteromorphisms and an increased risk for chromosomal and/or clinical anomalies including MR, has been suggested by several studies (Lubs and Ruddle, 1970; Nielsen et al, 1974; Jacobs et al, 1975; Boué et al, 1975; Halbrecht and Shabtai, 1976; Dutrillaux et al, 1980). In contrast, other studies have found no

differences in frequency of heteromorphisms between normal individuals and those with clinical abnormalities (Schwinger and Wehner, 1976; Iona et al, 1982) or MR (Lubs and Lubs, 1973; Lubs et al, 1977; Tharapel and Summit, 1978; Funderbunk et al, 1979).

Since chromosome heteromorphisms represent variability in chromosome structure (Verma and Dosik, 1980), it was suggested that the presence of variations could increase the risk of non-disjunction, malformation, and abortions (Patil and Lubs, 1977; Dutrillaux et al, 1980). For example, the rearranged genetic material in one such chromosome variant, a pericentric inversion of the centromeric region of chromosome three [inv(3c)], could predispose carriers to abnormality due to a position effect (Fogle and McKenzie, 1980). Inv(3c) has been reported in normal and mentally defective individuals at approximately the same frequency (4%) (Soudek and Sroka, 1979; Mikelsaar et al, 1978). Lin et al (1976) screened 980 neonates and found the inv(3c) at a frequency of 0.75%. It was concluded from these studies that inv(3c) heteromorphisms are not associated with MR or an abnormal phenotype more frequently than by chance. Likewise, no differences were found in the frequencies of other heteromorphisms between normal individuals and those with clinical abnormalities (Schwinger and Wehner, 1976; Iona et al, 1982) or MR (Lubs and Lubs, 1973; Lubs et al, 1977; Funderbunk et al, 1979).

The differences in the reported frequencies of chromosome heteromorphisms may be explained by a number of factors. These factors include:

1) Geographic variation. The incidence of the inv(3c) appears to be dependent on the study population as different geographic frequencies for the inv(3c) have been reported (Kaiser, 1984).
2) An age effect. A difference in the incidence of chromosome heteromorphisms has been found among neonates, 14-year-old children, and 65-year-old adults (Buckton et al, 1976).
3) Technical differences in cytogenetic staining methods. Chromosome heteromorphisms can vary both in size and staining affinity (Verma and Dosik, 1980). Technical differences in either staining procedures or estimation of size variations may result in a difference of reported incidences.
4) Population sample size.

At present, it is not certain whether or not chromosome heteromorphisms might have any clinical relevance.

C. Non-Specific X-linked Mental Retardation

1. X-linked Disorders

In diseases inherited on the X chromosome, the female is the carrier of the disorder. The heterozygous female will be partially or completely protected from the abnormal gene because one of her two X chromosomes is usually

inactive in every cell (Lyon hypothesis) (Lyon, 1961). Half of her sons will receive the X chromosome carrying the abnormal gene and will therefore be affected. Half of her daughters will be carriers.

Several specific biochemical or metabolic X-linked disorders are known to be associated with MR and distinct clinical phenotypes (McKusick, 1983). Two examples are Hunter's syndrome (a mucopolysaccharidosis characterized by severe MR) and the Lesch-Nyhan syndrome (a disorder of purine metabolism resulting in MR, muscle spasticity, and choreoathetosis, together with compulsive self-mutilation of lips and fingers). However, the majority of apparent X-linked disorders have no specific clinical stigmata and no detectable biochemical abnormality. This lack of physical anomaly has become characteristic of 'non-specific' X-linked MR (Turner et al, 1971).

2. Mental Retardation and X-linkage

A family with an apparently X-linked, non-progressive, form of mental deficiency was first described by Martin and Bell (1943). Affected family members had no apparent physical malformations and no motor or sensory dysfunction (Martin and Bell, 1943). Eleven males (severely retarded) and two females (mildly retarded) were found in two successive generations from this family. Incomplete penetrance of the X-linked gene was postulated to explain the presence of two apparently normal males with normal daughters who had

mentally retarded sons. Another kindred which showed a convincingly X-linked form of MR with no apparent clinical features was reported by Renpenning et al (1962): the mothers were mentally normal and about one half of their sons were mentally retarded. Many families with such X-linked MR have since been reported (Dunn et al, 1962; Opitz et al, 1965; Turner et al, 1970, 1972; Lehrke, 1972). This type of XLMR, with no specific physical abnormality, is generally referred to as non-specific XLMR.

Non-specific XLMR has been postulated to be the most frequent form of familial MR found in males (Turner et al, 1970; Lehrke, 1972; Davison et al, 1973; Turner and Turner, 1974). A 25-50% excess of males over females was found in institutions for the mentally retarded (Penrose, 1938; Lehrke, 1972) and Lehrke (1972) suggested the involvement of X-linkage. It was subsequently found that there were three times as many families with two affected sons than families with two affected daughters (Davison, 1973; Turner and Turner, 1974). The frequency of X-linked MR has been calculated to range from 0.53 (Turner and Turner, 1974) to 1.8/1000 (Herbst and Miller, 1980) in males and from 0.74 (Turner and Turner, 1974) to 2.4/1000 in females (Herbst and Miller, 1980). The difference in frequency between the two studies may be the result of a number of factors (Herbst and Miller, 1980). Turner and Turner (1974) studied only moderately retarded children who were attending school in a

10 year period. The exclusion of other levels of MR (mild, severe, profound) in this study may have resulted in an underestimation of the frequency for this disorder. Herbst and Miller (1980), on the other hand, reviewed cases which included all levels of MR. In addition, they ascertained probands (both living and dead) who had been diagnosed as mentally retarded in a 19 year birth cohort. This method of ascertainment would result in a more accurate determination of the incidence for non-specific XLMR. Nevertheless, these studies suggested that major genes relating to intelligence are located on the X chromosome, and that mutation in any of these genes could cause non-specific XLMR (Lehrke, 1972; Herbst and Miller, 1980).

3. The Fragile X Syndrome

Due to the lack of clinical stigmata, the diagnosis of non-specific XLMR is based on a family history of MR compatible with X-linkage. Recently, a cytogenetic marker has been found in association with some cases of non-specific XLMR (Lubs, 1969; Giraud et al, 1976; Harvey et al, 1977). This marker is a fragile X chromosome and, at present, represents the only clinical evidence which would confirm the diagnosis of non-specific XLMR.

a. Definition of a 'Fragile Site'

Fragile sites in human chromosomes were first described by Dekaban in 1965. These sites were later defined as areas of weakness at specific (non-random) points on chromosomes

(Hecht and Kaiser-McCaw, 1979). These weak points could be observed as breaks under certain culture conditions (see section C3c in Literature Review). Fragile sites have been observed on chromosomes 6, 7, 9, 10, 11, 12, 16, 20 and X (Figures 1, 3) (Moore et al, 1982). These sites are easily detected by light microscopy but their clinical significance remains uncertain. To date, only the fragile X has been shown to be associated with a human pathological condition, namely MR (Harvey et al, 1977; Sutherland 1979b).

The accepted criteria for a 'fragile site' were first described by Sutherland (1979a, b). They are as follows:

1) It is a non-staining gap of variable width usually involving both chromatids,
2) The site is always exactly at the same point on the chromosome,
3) The site is inherited in a Mendelian fashion, and
4) Fragility must be evident by the production (under appropriate in vitro conditions) of acentric fragments, deleted chromsomes, and triradial figures.

The difficulty in examining chromosomes for fragile sites is the occurrence of other chromatid gaps and breaks which can occur under the same cell culture conditions required for the expression of fragile sites (Glover, 1981; Sutherland, 1983). These gaps and breaks are referred to as autosomal lesions and are preferentially localized in bands 3q29, 6q26, 7q36 and 8q24 (Leversha et al, 1981; Soudek and

McGregor, 1981; Steinbach et al, 1982). These lesions are different from fragile sites because they do not meet the four criteria which define a fragile site. In addition, autosomal lesions rarely occur in more than 3-4% of metaphases from any individual whereas frequencies of the fragile X site usually range from 10-30% (Sutherland, 1983).

b. Discovery of the Fragile X Chromosome

In 1969, Lubs described a family with non-specific X-linked MR in which many members possessed an X chromosome with a constriction near the end of the long arm, resulting in a small knob separated by a thin stalk from the main portion of the chromosome (Figure 1). The thin stalk often appeared to be broken, as though it were "fragile". All male members of this family who possessed the fragile X were mentally retarded. The fragile X was also found at a lower frequency in the mother of two retarded brothers and the mother's sister. In one obligatory carrier (see section C3d in Literature Review) the marker was not found. This abnormal X, now designated fra(X)(q28), is the only cytogenetic marker that has been found in non-specific XLMR.

c. Mechanism of Induction

The presence of the fra(X) as a cytogenetic marker for non-specific XLMR was later confirmed in 1976 by Giraud et al and in 1977 by Harvey et al. The reason for the long lag period between the initial discovery and confirmation was provided by Sutherland (1977, 1979a) in a series of reports.

He found that the expression of the fragile site on the X chromosome was inhibited by the presence of folic acid and thymidine in the culture medium. Fra(X) expression was observed only if the folic acid content was low. This is generally accomplished by restricting fetal calf serum levels to a maximum of 5% in the culture media (Sutherland, 1979a; Howard-Peebles and Pryor, 1981). The culture media used by Lubs (1969) had a low folic acid content. This type of media subsequently became obsolete for use in the diagnosis of chromosome anomalies using banding techniques.

Fra(X) expression can also be enhanced by various agents which interfere with the normal metabolism of folic acid or directly inhibit thymidine biosynthesis (Figure 2). For example, fluorodeoxyuridine (FdU) prevents the formation of thymidine by inhibiting the enzyme thymidylate synthetase (Figure 2, #8) which catalyzes the conversion of deoxyuridine monophosphate (dUMP) to deoxythymidine monophosphate (dTMP) (Sutherland, 1979a; Glover, 1981; Tommerup et al, 1981; Gardner et al, 1983). Although FdU can induce fra(X) expression in media with inhibitory concentrations of folic acid, it cannot overcome the inhibitory effect of thymidine which is already present in the culture medium (Sutherland, 1979a; Glover, 1981; Gardner et al, 1983). Methotrexate and aminopterin also increase fra(X) expression by preventing folic acid from entering the available folate pool (Figure 2, #6) (Sutherland, 1979a;

Fonatsch, 1981; Mattei et al, 1981a). The inhibition of folate metabolism leads to a decrease of thymidine production (Figure 2).

There is no direct evidence yet to show either an in vivo folate deficiency (Popovich et al, 1982, 1983; Wang and Erbe, 1984) or a metabolic error in folate metabolism (Brondum-Nielsen et al, 1983a) in fra(X) patients. The mean corpuscular hemoglobin (MCH) in fra(X) patients was found to be at the upper end of the normal range (Langenbeck et al, 1984) whereas fra(X) negative patients with MR exhibited MCH levels at the lower end of the normal range. The suggestion that the fra(X) syndrome may be due to in vivo disordered folate metabolism (Langenbeck et al, 1984) is based on the indirect evidence that MCH levels are also increased in patients with folic acid deficiency diseases.

The postulated mechanisms for formation of the fra(X) are:

1) Specific alteration of DNA synthesis at chromosome region Xq28 (Giraud et al, 1976). The inhibition of fra(X) expression by thymidine suggested that impaired DNA replication was involved in fra(X) induction.
2) Deficient DNA-protein binding (Krumdieck and Howard-Peebles, 1983). It was suggested that any condition leading to impaired thymidine synthesis would promote misincorporation of uracil into DNA instead of thymidine. This could preclude a proper DNA-protein interaction and

result in a localized collapse of chromosome structure.

3) DNA methylation (Mixon and Dev, 1983; Daniel et al, 1984). The methylation of DNA base residues is known to prevent the transcription of the DNA sequence (Riggs and Jones, 1983). It has recently been shown that fra(X) expression can be reduced by 5-azacytidine, an inhibitor of DNA methylation.

d. Cytogentics of the Fra(X)

The fragile X chromosome is usually seen in approximately 10-30% of analyzed metaphases from affected males with MR (Sutherland, 1983). The detection of the fra(X) in carrier females, however, is much more difficult than in males. This can be demonstrated by the finding that the fra(X) chromosome is not always visible cytogenetically in mothers whose offspring are fra(X) positive. Since it is unlikely that spontaneous fra(X) mutations occurred in each affected offspring, these fra(X) negative mothers are defined as obligate fra(X) carriers (Sutherland, 1983). Carrier status in the female is thus determined by microscopic detection of the fra(X) and/or the presence of multiple fra(X) offspring if the fra(X) cannot be observed in the mother.

It first appeared that the proportion of metaphases showing the fra(X) decreased with advancing age in heterozygotes, especially in obligate carriers aged 30 years and above (Sutherland, 1977, 1979c; Turner et al, 1980b;

Jacobs et al, 1980). It was subsequently found that the incidence of the fra(X) decreased with increasing age only in mentally normal carriers. There was no age effect on fra(X) expression among mentally affected carriers (Howard-Peebles, 1980). Fra(X) expression in heterozygotes was shown to decrease as either their age or IQ increased (Jacobs et al, 1980). While several reports supported these correlations (Chudley et al, 1983; Schmidt, 1982; Fishburn et al, 1983), other studies found no association between the percentage of fra(X) cells, age, and mental performance in fra(X) heterozygotes Fryns and Van den Berghe, 1983; Filippi et al, 1983). A positive correlation between age and fra(X) expression in fra(X) males was also reported (Chudley et al, 1983). However, this finding was not confirmed (Brondum-Nielsen and Tommerup, 1984).

The conflicting data concerning the percentage of fra(X) expression in heterozygotes now appear to be the result of ascertainment bias in the relatives of mentally retarded male probands Uchida and Joyce, 1982). It was initially suggested that among older women, obligate carriers with multipl retarded sons were more likely to be screened cytogenetica ly for the fra(X) than were women who had no retarded sons (Uchida and Joyce, 1982). Uchida and Joyce (1982) found that the ascertainment by Turner et al (1980b) of retarded females rather than retarded males as index cases for the fra(X) failed to show an age effect

among carrier mothers of affected fra(X) females. The hypothesis of an ascertainment bias has now been substantiated by the report of a differential rate of reproduction between mentally normal and subnormal heterozygotes (Sherman et al, 1984a). A greater number of mentally normal carriers were found to have children than did mentally affected carriers (Fryns, 1984; Sherman et al, 1984a). The normal carriers would thus be detected at an older age when they had retarded sons or grandsons (Howard-Peebles, 1982b; Uchida et al, 1983).

An alternate hypothesis to explain the degree of MR found in heterozygotes is based on the finding that the fra(X) chromosome in a severely retarded fra(X) male with the Klinefelter syndrome was the early replicating (active) X chromosome (Froster-Iskenius et al, 1982). Other studies which attempted to demonstrate whether or not the fra(X) chromosome is preferentially the early replicating chromosome in affected heterozygotes show inconclusive findings. The fra(X) has been found to be the active X chromosome in the majority of cells from both mentally deficient (Howell and McDermott, 1982; Uchida and Joyce, 1982; Uchida et al, 1983) and normal carriers (Carpenter et al, 1982; Brondum-Nielsen et al, 1983b). An excess of inactive fra(X) chromosomes has also been reported in normal carriers (Brondum-Nielsen et al, 1983b; Knoll et al, 1984). Other investigations of mentally normal heterozygotes have

failed to show any evidence for preferential inactivation or activation of the fra(X) (Lubs, 1969; Martin et al, 1980; Jacobs et al, 1980).

These conflicting findings may be the result of a number of technical and theoretical considerations.

1) The method commonly used to distinguish between the active and inactive X chromosome is the incorporation of the thymine analog, 5-bromodeoxyuridine (BrdU) (Sutherland, 1983). When added to the cell culture at the end of the DNA replication (S) phase, BrdU is incorporated into the late replicating (inactive) X. The band at Xq28 is negatively staining and is difficult to detect with routine banding methods. BrdU incorporation further reduces the band's staining intensity. Scoring bias of the fra(X) may thus occur if solid staining is not first used to identify the fra(X) (Uchida et al, 1983). Such a scoring bias may have been present in the study of Carpenter et al (1982) (Uchida et al, 1983). In addition, BrdU is known to be cytotoxic and to generally decrease the percentage of cells expressing the fra(X) (Sutherland, 1979a; Jacobs et al, 1980; Uchida and Joyce, 1982; Brondum-Nielsen et al, 1983b).

2) Fra(X) expression has been reduced following co-cultivation (Eberle et al, 1981, 1982) and hybridization (Bryant et al, 1983) between normal and fra(X) cells.

This metabolic cooperation may impair a correct interpretation of fra(X) frequency in uncloned heterozygote cells which differ with respect to their X-inactivation status (Steinbach et al, 1983a).

3) The lack of precise IQ evaluations in fra(X) heterozygotes may preclude an accurate correlation between the replication pattern of the fra(X) and mental capacity (Paul et al, 1984). It has been shown that many apparently 'normal' carriers may be dull or mildly retarded when subjected to actual psychometric evaluation (Paul et al, 1984). Such IQ inaccuracies would result in erroneous correlation between inactivation of the fra(X) and mental status.

4) The frequency of the fra(X) expression may be underestimated due to loss of the chromosome material to the fragile site (Fitchett and Seabright, 1984). It is not known whether this loss occurs during culture or as a result of the slide-making procedure.

5) The majority of studies on fra(X) inactivation in heterozygotes have been performed on peripheral lymphocytes. These studies are complicated by reports of variable replication patterns of the inactive X chromosome in human peripheral lymphocytes (Schmidt et al, 1982; Schmidt and Stolzman, 1984). It has been shown that although the initiation time of DNA synthesis in the inactive X is constant, termination of synthesis can

occur later, earlier, or at the same time as the active X (Schmidt and Stolzman, 1984). It is not known whether this altered replication cycle is transmitted to all daughter cells or whether this diverse replication pattern has any functional significance (Schmidt and Stolzman, 1984). However, such an allocyclic fra(X) chromosome could give misleading results in inactivation studies of the X chromosome.

The only conclusive study regarding fra(X) expression and mental status was reported by Steinbach et al (1983a,b). Fibroblast clones from two female fra(X) heterozygotes, one mentally retarded and one mentally normal, showed that cell cultures consisted of two subpopulations (Steinbach et al, 1983a): 1) cells which expressed the fra(X) and 2) cells in which the fra(X) could not be induced even through the application of FdU. Cells from the mentally retarded heterozygote showed the fra(X) in the majority of clones. The majority of clones from the mentally normal heterozygote, however, did not show the fra(X). In another study, all the fibroblast clones from an affected fra(X) hemizygote were demonstrated to carry the fragile site (Steinbach et al, 1983b). These results substantiate the hypothesis of a correlation between the frequency of fra(X) cells and mental development in heterozygotes. Based on the Lyon hypothesis (Lyon, 1961), it can be predicted that the degree of mental impairment would be related to the

proportion of cells with the fragile site on the active X.

e. Segregation Analysis

i) Pedigree Information

An analysis of family pedigrees of the fra(X) syndrome failed to reveal a pattern that was consistent with classical X-linkage (Sherman et al, 1984b; Howard-Peebles and Friedman, 1984). The presence of the fra(X) has been reported in unaffected males who apparently transmitted the disorder via their daughters to their affected grandchildren (Daker et al, 1981; Daker, 1983; Webb et al, 1981a; Brondum-Nielsen et al, 1981; Fryns and Van den Berghe, 1982, 1983; Rhoads et al, 1982; Veenema and Geraedts, 1983). The initial non-specific XLMR pedigree reported by Martin and Bell in 1943, now known to be fra(X) positive (Richards et al, 1981), also suggested transmission through apparently normal males.

In families with X-linked disorders, the expected ratio of females:affected males:normal males is 2:1:1 (Herbst and Miller, 1980). However, a 20% deficit of mentally affected fra(X) males has been reported recently (Sherman et al, 1984a). It was suggested that this deficit was due to non-penetrance of the fra(X) 'gene', resulting in apparently normal males who could then transmit the fra(X). A lack of sporadic cases of fra(X) males has also been reported in the same study (Sherman et al, 1984a).

In a classical X-linked disorder, it is assumed that

affected males do not reproduce and that mutation rates are equal in males and females (Haldane, 1935). In order to maintain the X-linked gene in population equilibrium, 1/3 of all affected males in each generation must be the result of fresh mutations (sporadic cases) (Haldane, 1935). This is necessary in order to replace 1 of the 3 X chromosomes (2 in the female and 1 in the male) lost in each generation due to non-reproducibility of the affected males (Haldane, 1935). The lack of sporadic fra(X) males suggested that fra(X) mutations did not occur in maternal oocytes and that all fra(X) mutations must therefore occur in the sperm (Sherman et al, 1984a). The mutation rate was estimated to be 7.2×10^{-4} (assuming a single locus with Mendelian inheritance), an order of magnitude greater than those in other X-linked disorders (Sherman et al, 1984a).

Heterozygosity for the fra(X) has been found in 56% of females from fra(X) families (Sherman et al, 1984a). Of these carrier females, 30% had some degree of mental impairment (Sherman et al, 1984a). In addition, it was determined that approximately 1/2 of fra(X) heterozygotes were fresh mutations and that no males with XLMR would be observed among their brothers (Sherman et al, 1984a). The same finding has been reported for heterozygotes with the Lesch-Nyhan syndrome (Francke et al, 1976) and fra(X) negative non-specific XLMR (Herbst and Miller, 1980).

Recent studies (Sherman et al, 1984b; Howard-Peebles

and Friedman, 1984) have shown that the obligate carrier mothers and daughters of unaffected fra(X) males are rarely, if ever, mentally impaired and that the sibs of these transmitting males are much less likely to be retarded than the sibs of mentally impaired males. It was suggested that the fra(X) 'gene' may be silenced, perhaps as a consequence of meiosis in the carrier mother (Lubs, 1984, personal communication).

ii) Linkage Studies

Fra(X) transmission through an apparently unaffected male was demonstrated by studies on the linkage between the fra(X) and the closely linked haemophilia B (factor IX) gene (Camerino et al, 1983). However, Choo et al (1984) have reported that the linkage between the fra(X) and factor IX was too loose to be of clinical value. More conclusive determinations concerning fra(X) transmission may be gained through the use of closely linked restriction fragment length polymorphisms (RFLPs) (Drayna et al, 1984).

Restriction fragment length polymorphisms are visualized as a result of base sequence variation in the DNA. RFLPs are produced by enzymes (restriction endonucleases) which break the DNA double helix at a variety of specific base sites (Mange and Mange, 1980). RFLPs are being used to construct a complete linkage map of the human genome and are thus potentially useful as diagnostic markers of genetic disease (Wieacker et al, 1984). Any new disease

locus can then be localized with respect to these markers (Aldridge et al, 1984). Close linkage of three such RFLPs, termed marker 52A (Drayna et al, 1984) and ST14 and DX13 (Holden, 1984, personal communication), to the fra(X) site has recently been demonstrated. Linkage between these markers and the fra(X) site revealed carrier status in a female who was considered to be fra(X) negative (Holden, 1984, personal communication). These markers can also be used for determining fra(X) transmission through apparently normal males.

Conventional linkage analysis between the fra(X) and the X-linked enzyme, glucose-6-phosphate dehydrogenase (G6PD), suggests that the chromosomal region near or at band Xq28 exhibits a very high rate of meiotic exchange (Filippi et al, 1983; Howard-Peebles and Carroll, 1984). If a gene related to intelligence does exist in band Xq28, the high rate of recombination at this site may be associated with the high mutation rate of the fra(X) gene (Turner and Jacobs, 1984).

f. Fra(X) and Other Chromosome Abnormalities

There have been several reports of an increased incidence of the fra(X) in individuals who also have other major chromosome abnormalities: XXY (Wilmot et al, 1980; Froster-Iskenius et al, 1983; O'Brien et al, 1984); XY/XXY (Fryns et al, 1983); XYY (Sutherland, 1983); Down syndrome (Jacobs et al, 1980; Filippi et al, 1983); and inversions

and translocations (Howard-Peebles, 1982a, Willey et al, 1983). Fra(X) females have been shown to have a higher incidence of hyperdiploid cells than control females without the fra(X) (Kahkonen et al, 1983). Hecht et al (1983) reported a female with the Turner syndrome (45,X) who also had the fra(X) in addition to a Robertsonian translocation of the #13 chromosomes. The daughter of an obligate fra(X) carrier had a 47,XXX karyotype (Kahkonen et al, 1983). A fra(X) heterozygote was observed to have an interstitial deletion in her fra(X) chromosome, del fra(X)(q22 - q26) (Kaiser-McCaw and Hecht, 1980).

It has been suggested that "stickiness" at the fragile site could lead to malsegregation during meiosis or mitosis (Kahkonen, 1983). Similar associations between other fragile sites and chromosome abnormalities have also been reported (Garcia-Sagredo et al, 1983; Sessarego et al, 1983; Mules et al, 1983). The association of fragile sites with chromosome anomalies may, however, be the result of ascertainment bias (Sutherland, 1983). The examination of chromosomally abnormal individuals as index cases rather than patients with fragile sites may fail to show a preferential association of fragile sites with chromosomal abnormalities.

g. Incidence of the Fra(X)

The frequency of the fra(X) chromosome in the general population is still unknown. Only one neonatal survey for

the fra(X) has been reported to date. Of 1,019 unselected neonates examined (522 males, 497 females), no cases of the fra(X) were identified (Sutherland, 1982). Kison et al (1982) surveyed all moderately and severely retarded males in a rural Swedish population. The fra(X) was found at a frequency of 0.4/1000 males. This value may be an underestimation due to the exclusion of mildly retarded males (Kison et al, 1982).

It has been estimated that approximately 1/3 to 1/2 of XLMR in males may be due to the fra(X) (Turner et al, 1980a; Herbst and Miller, 1980; Mattei et al, 1981a). Based on a frequency of 0.53-1.8/1000 males with XLMR (Turner et al, 1980a; Herbst and Miller, 1980), the frequency of the fra(X) can be estimated to range from 0.19-0.92/1000 liveborn males (Herbst and Miller, 1980; Fishburn et al, 1983).

Fra(X) screening of the institutionalized males has revealed frequencies ranging from 0.9-9.2% (Venter and Op't, 1982; Carpenter et al, 1982; Sutherland, 1982; Blomquist et al, 1982, 1983; Jacobs et al, 1983; Froster-Iskenius et al, 1983). The wide range reported in the aforementioned studies may be a result of the different selection criteria (Kahkonen et al, 1983) that were used in different studies, such as: (1) MR at any or all levels (mild, moderate, severe, profound), (2) physical phenotype, and (3) family history of XLMR (discussed further in section D3c in Literature Review).

The contribution of the fra(X) chromosome to mental handicap in females remains uncertain. If the prevalence of the fra(X) in males is approximated to be 0.5/1000, it implies that the female heterozygote frequency is 1/1000 (Turner and Jacobs, 1984). It has been determined that approximately 30% of fra(X) carriers manifest some degree of mental impairment (Sherman et al, 1984a; Turner and Jacobs, 1984). The frequency of expressing heterozygotes for the fra(X) was estimated to be 0.2-0.4/1000 females (30% of 1/1000) (Fishburn et al, 1983; Turner and Jacobs, 1984). Fra(X) screening of mildly retarded females has yielded frequencies of 4% (5/128) (Turner et al, 1980b) and 0% (0/61) (Blomquist et al, 1983).

D. Testicular Size and Mental Retardation

Abnormal testicular volume has been reported to be a common physical feature in males with MR syndromes. Small testes are often found in karyotypically abnormal males (reviewed by Summitt, 1979). Enlarged testes have recently been reported in association with non-specific XLMR, including the fra(X) syndrome (Turner et al, 1978; Herbst and Miller, 1980; Fishburn et al, 1983). Thus, testicular size can be a useful clinical marker in the characterization of chromosome abnormality syndromes.

1. Normal Testicular Development

Testicular volumes of pre-adolescent boys are relatively constant until puberty and they do not usually exceed 1.7 ml (Laron and Zilka, 1969; Cassorla et al, 1981). The genitalia usually begin to develop between the ages 9 1/2-13 1/2 (mean 11.6) years in 95% of boys and reach maturity at ages varying between 13-17 (mean 14.9) years (Marshall and Tanner, 1970). Early maturational changes in the testis are found in the intertubular connective tissue and are associated with the transformation of mesenchymal cells into immature Leydig cells (August et al, 1972). These changes are followed by the development of the Sertoli cells, and spermatogenesis to the spermatozoa stage (August et al, 1972). Testicular volume increases from a prepubertal value of 1.7 ml to an average adult volume of 17-19 ml (Laron and Zilka, 1969; Boisen, 1979; Farkas, 1976; Zachmann et al, 1974). The 90th and 10th adult percentiles are 22 and 12 ml respectively (Zachmann et al, 1974).

2. Determination of Testicular Volume

Testicular volume has been evaluated by two methods: (1) orchidometer and (2) sliding calipers.

An orchidometer is a series of graded, egg-shaped models of a known volume. Testicular volume is estimated during palpation by comparing the patient's testis with a testicular model of known volume (Daniel et al, 1982).

However, orchidometers are not standardized and volume determination by subjective palpation may lead to error (Daniel et al, 1982).

Actual measurements of testicular length and width can be made with sliding calipers or a ruler (Daniel et al, 1982). These measurements can be affected by the shape of the testis, relationship of the epididymis to the testis, layers of scrotal skin and the compression of tissues (Daniel et al, 1982). If measurements of testicular length and width are available, then the volume can be calculated.

Different formulas based on testicular shape have been used to calculate testicular volume (Daniel et al, 1982). The testis has been considered to be a prolate spheroid and the empirical formula used to determine testicular volume (V) was: $V = 0.71 \times l \times w^2$ (Lambert, 1951). Some investigators regard the testis as an ellipsoid and calculate volume according to the formula $V = \frac{\pi}{6} \times l \times w^2$ (Cantu et al, 1976). The volumes determined by the orchidometer showed a higher concordance with the volumes calculated by using the formula of Cantu et al (1976) than those calculated using the formula of Lambert (1951) (Daniel et al, 1982; Howard-Peebles and Finley, 1983). However, testicular volumes determined by the formula of Cantu et al (1976) and the orchidometer can still differ in the same individual (Brondum-Nielsen et al, 1982).

Testicular development, as determined by various investigators, is summarized in Figure 7. The greatest increments in testicular enlargement occurred between 13-15 years (Figure 7). Testicular growth during puberty reaches a peak by 17 years (Figure 7).

3. Abnormal Testicular Size

a. Hypogonadism

i) Testicular Pathology

Male hypogonadism is a condition characterized by decreased functional activity of the gonads which leads to retardation of sexual development and reduced testicular volume (micro-orchidism) (Jannsen, 1962).

The etiology of hypogonadism includes: (1) testicular causes, both congenital (chromosomal abnormalities, neurological disorders) and acquired (chronic liver disease, alcoholism, mumps orchitis, irradiation, chemotherapy); (2) male pseudohermaphroditism; and (3) disorders of the pituitary and hypothalamus (Killinger, 1979; Paulsen, 1974).

A number of karyotype abnormalities in the male are associated with hypogonadism. These chromosomal anomalies include:

(1) 47,XXY. Hypogonadism is a common symptom observed in individuals with the Klinefelter syndrome. Testicular volumes in these individuals do not usually exceed 2.5 ml. Testicular histology of XXY newborn infants appears to be

normal. However, there is a gradual loss of germ cells during childhood. During puberty, there is a progressive hyalinization of the seminiferous tubules and clumping of the Leydig cells (Killinger, 1979).

(2) 47,XYY. The majority of males with the XYY karyotype have normal gonadal function but a small number of these individuals have impairment of both spermatogenesis and testosterone production. Testicular biopsy specimens obtained from XYY patients with impaired gonadal function show either absence of germ cells or a variable range in gametogenic impairment (Paulsen, 1974).

(3) 47,XY,+21. Down syndrome males generally demonstrate reduced testicular size (Summitt, 1979). Puberty is delayed and deficient and patients are sterile (Sergovich, 1976).

(4) 46,XX. Although not a chromosomal male, the XX male is generally characterized by small testes and hypogonadism (Summitt, 1979). Histologically, the testes show decreased size and numbers of seminiferous tubules, absence of germ cells, peritubular and interstitial fibrosis, and hyperplastic Leydig cells.

(5) Balanced translocations. Hypogonadism has also been found in infertile men with apparently balanced translocations (Chandley et al, 1976; Handelman and Smith, 1983). The immediate cause of sterility appears to be a variable degree of gametogenic impairment. This impairment results in severe oligospermia or azoospermia.

(6) Other. A wide variety of other autosomal abnormalities, mainly duplication and deletion anomalies, have also been found in association with male hypogonadism (Summitt, 1979).

Well established neurogenic causes of hypogonadism include inflammatory or traumatic lesions of the spinal cord (Reichlin, 1974). Approximately 50% of males with paraplegia sustained by trauma show some degree of decreased testicular function (Paulsen, 1974). This decreased function is the result of spermatogenic arrest due to hypoplasia of the germinal epithelium. Leydig cells can be normal, but hyperplasia has been reported. No disturbance of sex hormone production has been found (Reichlin, 1974).

Hypogonadism has also been found in a large percentage of males with neurological or psychiatric illness, including MR (Vague et al, 1963; Bostrum and Brun, 1971; Sylvester, 1973). A marked correlation was found between hereditary cerebellar ataxias and gonadal/genital disorders (Neuhauser and Opitz, 1975). It was suggested that cerebellar or cerebral lesions might result in disturbed stimulation of the testes (Bostrum and Brun, 1971; Sylvester, 1973). Abnormal testicular histology included azoospermia, reduced spermatogenesis, Leydig cell aplasia and hyperplasia, and tubular and interstitial fibrosis (Sylvester, 1973).

Hypogonadism can also occur as a consequence of disturbed levels and functions of the sex hormones. These

disturbances may be caused by:

(1) Diseases of the pituitary or hypothalamus (tumors, meningiomas, encephalitis, and demyelinating disorders). These disorders can cause a deficiency of sex hormone production (Reichlin, 1974).

(2) Male pseudohermaphroditism. This is a group of disorders characterized by a normal 46,XY karyotype and a variable degree of sexual ambiguity caused by abnormalities in sex hormone function and target organ response (Summitt, 1979).

(ii) Cryptorchidism

The incidence of undescended testes in liveborn males is about 10% (Paulsen, 1974). By the end of the first year, the incidence of undescended testes is approximately 2% (Killinger, 1979; Paulsen, 1974). This incidence drops to less than 1% by puberty (Killinger, 1979). Approximately 0.3-0.4% of post-pubertal males have either unilateral or bilateral undescended testes (Paulsen, 1974). Failure of one testis to descend occurs 4-5 times as frequently as bilateral maldescent (Killinger, 1979).

The frequency of bilateral cryptorchidism among institutionalized adult males has been reported to be 0.4% (Cassiman et al, 1975). Cryptorchidism can often be found in chromosomal syndromes commonly associated with hypogonadism (Paulsen, 1974).

(iii) Incidence of Hypogonadism

It is generally accepted that 0.6% of adult males in the general population have hypogonadism, (Johnsen, 1962; Talbot et al, 1955). The incidence of hypogonadism among institutionalized males has not been examined extensively.

Published studies on the incidence of hypogonadism among MR males have been based on subjective interpretations of 'hypogonadism.' Cassiman et al (1975) reported that 1.9% of retarded adult males had hypogonadism (a testis smaller than 4 X 2 cm) or cryptorchidism. These measurements are equal to a testicular volume of 8.32 ml, according to the formula of Cantu et al (1976). Nielsen and Fischer (1965) described one or more 'hypogonadal' signs (small, soft testes of prepubertal size, feminine fat distribution, mammae development, scanty beard growth) in 3.3% of mental retardates examined. Sex chromosomal abnormalities were found in 30.4% of these 'hypogonadal' males. Autosomal anomalies were detected in 3.5%.

A specific testicular volume has not been clinically established for micro-orchidism. Rundle and Sylvester (1962) suggested that a testicular volume $\leq$12 ml, or 2 S.D. below their reported mean of 31.1 $\pm$ 9.5 ml for institutionalized MR males, be used as a clinical indication for hypogonadism. However, as their volumes were calculated according to the formula of Lambert (1951) and far exceeded previously reported values (Figure 7), this limit for micro-

orchidism has not been generally accepted.

Baker et al (1975) defined micro-orchidism as a testicular volume $\leq$ 15 ml. Their definition was based on the observation that only 5% of males in the 21-50 yr age group had testicular volumes $\leq$ 15 ml as compared to 27.5% of males over the age of 50 yr.

b. Testicular Enlargement

i) Etiology

Several conditions may result in bilateral enlargement of the testes. These conditions include:

(1) Benign macro-orchidism. Bilateral testicular enlargement, unassociated with any pathologic process, has been described in normal adult males (Nisula et al, 1974; Padron et al, 1979). In a survey of 202 normal males (19-49 yr), 15 (7.4%) were found with benign macro-orchidism (Padron et al, 1979).

Testicular volume in these macro-orchid male subjects exceeded the 95% confidence limits (V $\geq$25 ml) for a normal population (Zachmann, et al, 1974; Nisula et al, 1974; Padron et al, 1979; Brown et al, 1981). The testes were enlarged symmetrically with no visible or palpable abnormalities. Semen analysis was normal (Padron et al, 1979). Testicular biopsies showed: (a) an increase in the number of seminiferous tubules which had few mature germinal elements, (b) spermatogonia with ballooned cytoplasm, and (c) Sertoli cells with retracted cytoplasmic apices (Nisula

et al, 1974). Endocrinologic evaluation in these patients was normal (Nisula et al, 1974).

2) Idiopathic or cerebral precocious puberty and juvenile hypothyroidism. In precocious puberty, the testicles are bilaterally enlarged in relation to the age of the patient. However, the enlarged volume is appropriate for the patient's advanced pubertal stage of development (Breen et al, 1981). Similar to idiopathic puberty, severe juvenile hypothyroidism may be associated with severe precocity and enlargement of both testes (Laron et al, 1970).

3) Adrenal rest tumors. These tumors are composed of hypertrophic adrenal rest tissue and usually cause asymmetrical nodular masses in the testes. Precocious virilization is usually present (Newell et al, 1972).

4) Bilateral testicular neoplasm. Patients with bilateral testicular malignancies generally have asymmetrical enlargement of each testicle (Breen et al, 1981).

5) Non-specific XLMR. Escalante et al (1971) first described a family with non-specific XLMR in which affected males had enlarged testes. This study was followed by several reports on the association of benign macro-orchidism with some cases of non-specific XLMR (Turner et al, 1972, 1975; Cantu et al, 1978; Ruvalcaba et al, 1977). Following the rediscovery of the fra(X) (Giraud et al, 1976; Harvey et al, 1977), a study by Turner et al (1978) showed that benign macro-orchidism was associated specifically with fra(X)

positive males. Subsequent studies showed contradictory findings. While many males with the fra(X) were found with macro-orchidism (Sutherland, 1979c; Sutherland and Ashforth, 1979; Howard-Peebles and Stoddard, 1979, 1980; Jacobs et al, 1979, 1980; Turner et al, 1980a; Martin et al, 1980; Howard-Peebles, 1980; Gustavson et al, 1981; Richards et al, 1981; Carpenter et al, 1982; Webb et al, 1982; Brondum-Nielsen et al, 1983a), not all fra(X) positive males were macro-orchid (Sutherland and Ashforth, 1979; Jacobs et al, 1979; Richards et al, 1981; Proops and Webb, 1981; Webb et al, 1982). A correlation of macro-orchidism within fra(X) families also yielded inconsistent findings: some affected males had enlarged testes but their affected brothers did not. Thus, non-specific XLMR with macro-orchidism can be divided into two categories: fra(X) positive and negative (Fishburn et al, 1983). However, micro-orchidism ($V = 12.8 \pm 8.1$ ml) has also been described (Fox et al, 1980) in affected males from the non-specific XLMR family which was reported originally by Renpenning et al in 1962 (see section C2 in Literature Review). This family has since been screened for the fra(X) chromosome and has been reported to be fra(X) negative (Fox et al, 1981).

Endocrine studies in macro-orchid fra(X) positive and negative males with non-specific XLMR have shown no consistent endocrine abnormality (Turner et al, 1975; Ruvalcaba et al, 1977; Cantu et al, 1976; Bowen et al, 1978; Breen et al,

1981; Brondum-Nielsen et al, 1982). Testicular biopsy specimens from these patients have also demonstrated no consistent pathological features (Turner et al, 1975; Ruvalcaba et al, 1977; Bowen et al, 1978; Cantu et al, 1978).

6) Other. Benign processes such as compensatory hypertrophy of the contralateral testicle in boys with unilateral cryptorchidism may be associated with gonadal enlargement in the male (Laron and Zilka, 1969). Various pathological processes such as hydrocele, varicocele or tuberous sclerosis may also result in dramatically enlarged testes.

(ii) Incidence of Macro-orchidism

A testicular volume $\geq$ 25 ml is defined as the 95th percentile in a normal male population (Zachmann et al, 1974). The incidence of macro-orchidism (V $\geq$ 25 ml) among institutionalized MR males has been reported to be 20-29% (Pozsonyi et al, 1981; Brondum-Nielsen et al, 1982; Howard-Peebles and Finley, 1983). Volumes exceeding 25 ml (V > 25 ml) have been found in 3.4-6.0% of MR males (Cantu et al, 1976; Brondum-Nielsen et al, 1982; Shapiro et al, 1983). Extremely enlarged testes (V > 34 ml) were reported in 8.3% of MR males examined (Howard-Peebles and Finley, 1983).

The incidence of macro-orchidism among males with non-specific XLMR has not been examined extensively. Only one preliminary study has been reported to date. Of 15 males with non-specific XLMR, 5 (33%) were found to have a volume

greater than 25 ml. Four of these 5 macro-orchid males (80%) had the fra(X) (Brown et al, 1981).

The incidence of the fra(X) among MR males who had testicular volume $\geq$ 25 ml was found to be 4% (Brondum-Nielsen et al, 1982). This incidence increased to 36-60% if males with testicular volumes exceeding 25 ml were screened (Brondum-Nielsen et al, 1982; Shapiro et al, 1983). The incidence of the fra(X) among institutionalized males with a testicular volume > 34 ml was 27% (Howard-Peebles and Finley, 1983). The observed fra(X) frequency in macro-orchid males was thus found to be highly dependant on the degree of testicular enlargement.

III Materials and Methods

A. Materials

1. The Study Group

Approximately 1,000 mentally retarded (excluding Down syndrome) males were made available for study from four Ontario institutions: 1) Southwestern Regional Centre, Blenheim, Ontario; 2) Oxford Regional Centre, Woodstock, Ontario; 3) Huronia Regional Centre, Orillia, Ontario; and 4) St. Thomas Adult Rehabilitation and Treatment Centre, St. Thomas, Ontario. Consent was obtained for 878 of these residents to have testicular measurements performed. Testicular length and width were measured by a medical doctor using sliding calipers. Blood samples for cytogenetic analyses were drawn by registered nurses and technologists.

Adult residents ($\geq$ 18 years) were grouped into three categories based on their testicular volume (V): 1) macro-orchidism ($V \geq 25$ ml), 2) micro-orchidism ($V \leq 15$ ml), 3) normal ($15 < V < 25$ ml). All levels (mild, moderate, severe, profound) of MR were included. Cytogenetic studies, both for the fra(X) and conventional chromosome abnormalities, were conducted on all adult residents with macro-orchidism. An equivalent number of adult males with micro-orchidism and normal testicular volume, selected by means of a random numbers table, were also examined cytogenetically. All fra(X) screening was performed by the

author. Routine cytogenetic analysis by quinacrine fluorescence was done in co-operation with the technical staff at the Cytogenetics Laboratory, CPRI, London, Ontario. Special staining techniques (G-, R-, C-, NOR-, and methyl green/DAPI C-banding) used to characterize chromosome abnormalities were performed by the author. Following chromosome studies, the case histories of the residents belonging to the three groups of testicular volume were reviewed by the author for height, presence of familial retardation and etiological classification of MR.

The family pedigrees of the karyotypically normal residents were screened for the presence of possible fra(X) negative non-specific XLMR. Pedigrees were postulated to show XLMR if they conformed to one of the following criteria (Herbst and Miller, 1980; Herbst, 1980; Fishburn et al, 1983):

1) affected males were present in at least two generations related through the mother of the proband;
2) affected males were present in two sibships whose mothers were sisters; and
3) at least two mentally retarded males were present in the proband's sibship (singly affected sibship).

Pedigrees which showed positive family history of MR that did not conform to X-linkage were defined as non-specific familial MR (Herbst and Miller, 1980). Pedigrees were excluded from the X-linkage and non-specific familial

category if the proband's MR was recorded as being due to: infections and intoxications; trauma or physical agents; disorders of metabolism, growth, and nutrition; gross postnatal brain disease; chromosomal abnormalities; and major psychiatric disorders (Herbst and Miller, 1980). The remaining pedigrees were listed as MR of unspecified etiology if the proband's MR was recorded as due to unknown cause.

The patients who were cytogenetically screened were then grouped according to the scheme in Table 6:

1) conventional chromosome abnormalities (Table 7);

2) non-specific XLMR, both fra(X) positive and negative (Table 8);

3) other genetic factors, such as non-specific familial MR, consanguinity, autosomal dominant and recessive disorders, and syndromes of unknown etiology (Table 9);

4) environmentally caused, including MR due to pre-natal and post-natal disease, trauma and miscellaneous (lead poisoning, hypothyroidism, major psychiatric disorders) (Table 10); and

5) MR of no specified cause (Table 10).

2. Statistical Analysis

a) Difference Between Two Success Probabilities

Statistical analyses on the frequencies presented in Table 6 were performed using a two-sided Z-test for the

difference between two success probabilities ($\hat{p}_i$) (see Appendix 2). In order to test for a difference among three $\hat{p}_i$'s (distribution of males with a specific disorder in the three testicular volume groups), the Z-test was performed three times for each disorder (Table 23). This was done to test for statistical difference in distribution among 3 sets of residents: 1) macro-orchid (p_1) and micro-orchid (p_2), 2) micro-orchid (p_2) and normal testicular volume (p_3), and 3) macro-orchid (p_1) and normal testicular volume (p_3) (Table 23). In order to test for a difference between three p_i's at an overall significance of $\alpha = 0.05$, the Z-scores for each set of residents (Table 23) were tested at a significance level of $\alpha = 0.017$ (0.05/3).

b) Stratification Random Sampling

The overall frequencies (P_o) of the various disorders in the total adult population of institutionalized males were calculated retrospectively by statistical stratification (see Appendix 2). This was done to account for the different proportions of non-Down syndrome institutionalized males found in each testicular volume group. The overall frequencies (P_o) (Table 20) can be applied only to the original group as the actual distribution of testicular volume among all institutionalized males is not known.

However, as there is no reason to suppose that the distribution of testicular volume in the present study is not a representative sample, the calculated overall frequencies may be extended to all institutionalized non-Down syndrome males.

3. Family Studies

When a chromosome abnormality was found, familial studies were initiated in order to trace the origin of the defect as well as to offer genetic counselling to family members. In the case of fra(X) positive families, an extended effort was made by the clinical staff to contact all female relatives to inform them of their possible carrier status. All family follow-up cytogenetic studies were conducted by the author.

B. Methods

1. Determination of Testicular Volume

All testicular measurements were done with sliding calipers. The scrotal skin was gently pulled tight against the testicle and the length (l) and width (w) measured. Testicular volume (V) was calculated according to the formula $V = \frac{\pi}{6} \times l \times w^2$ (Cantu et al, 1976). For the sake of expediency for the hospital staff, only the right testis was measured unless an obvious size difference was noted. Both testes of fra(X) positive males were later re-measured to confirm the testicular volume (Table 25). Although some variability existed between the two measurements, the

differences did not alter the placement of males among the three testicular volume groups.

The average volume and one standard deviation for each age group was calculated and plotted (Figure 6).

2. Culture Methods

Under aseptic conditions, 10 ml of venous blood was obtained from each patient. Five ml were drawn into each of a heparinized and non-heparinized vacutainer tube (Beckton, Dickinson & Co., Canada Ltd., 2464 South Sheridan Way, Mississauga, Ontario, L5J 2M5 - green stopper #6483, red/gray stopper #6511). These tubes were allowed to stand until the white blood cells had settled in the former and the blood clotted in the latter. Approximately 2.0 ml of the heparinized cell-rich supernatant was added to culture bottles containing 10 ml of chromosome media 199 [Grand Island Biological Company (GIBCO), Grand Island, New York, U.S.A. 14070] with phytohemaglutinin (Wellcome Reagents Ltd., Beckenham, England, BR3 3BS) (45 mg/5 ml), an antibiotic/ antimycotic mixture (GIBCO; penicillin 100,000 units/ml; fungizone, 25 g/ml; streptomycin, 10,000 g/ml) (1 ml to 100 ml media) and heparin (GIBCO, 200 units/ml) (1 ml to 100 ml media). This gave an initial white blood cell concentration of approximately 2000 leukocytes per ml of medium. All the serum from the non-heparinized vacutainer was added to the culture media. If insufficient patient serum was available, human AB serum (courtesy of Victoria Hospital, London, Ontario) was used. The cultures were incubated at 37°C for 96 hr.

Colcemid (0.3 ml) (Gibco, 10 µg/ml) was added for the last two hours.

A fragile X chromosome was suspected if either a chromatid or chromosome break or secondary constriction was noted near band Xq28 (Figure 4).

3. Standard Metaphase Preparation

Following hypotonic treatment with 0.075 M KCl at 37°C for 12 minutes, the cells were fixed and washed three times with fresh, cold acetic acid-alcohol (3:1 methanol:glacial acetic acid). The cell suspension was dropped from a height of approximately 6 inches onto clean, glass microscope slides. The glass slides were then flamed, placed in a vertical position and allowed to dry at room temperature.

4. Scoring of Fragile Sites

The slides were stained in 5% Giemsa (Gurr's R66) in Sorensen's phosphate buffer (pH 6.8) for 10 minutes. Slides were then rinsed twice in distilled water and allowed to air dry thoroughly at room temperature.

Fifty complete metaphase spreads were examined for each male patient and 200-300 for every female patient. The co-ordinates of the microscope stage position of all metaphase figures with telomeric breaks or constrictions were recorded. The identity of these chromosomes was later established using quinacrine fluorescence (Figures 4 and 5).

A diagnosis of fragile X was made only after the break or constriction on Xq28 was confirmed in two consecutive cell cultures (Table 17).

5. Quinacrine Fluorescence

a) Staining procedure (Sergovich, 1976)

Three gm of quinacrine dihydrochloride (Sigma Chemical Co., P.O. Box 14508, St. Louis, Missouri, U.S.A. 63178) was dissolved in 50 ml of 1:1 glacial acetic acid:propionic acid in a Coplin jar. The slides were dipped into the mixture, immediately removed and placed in a dry Coplin jar. The jar was rapidly filled with running cold water and immediately emptied. This was repeated five or six times. The slide was then rinsed in, and mounted with a phosphate buffer at a pH of 5.8.

b) Microscopic and Photographic Procedures

For viewing quinacrine fluorescent banding, a Zeiss microscope with an HBO 50 W high-pressure mercury light source was used. Photographs were taken using KODAK technical pan film 2415 (Estar-AH base) through a Planapo 63 X (Zeiss) objective with oil immersion and a 1.6 OPTOVAR lens. An exposure time of 10-15 seconds was used, depending on the fluorescent intensity of the particular chromosome spread. The negatives were developed with Kodak D-19 for five minutes at 20°C. Prints were made on Kodak polycontrast rapid II RC paper and enlarged to 8 X 10 inches.

c) Chromosome examination

All telomeric breaks or constrictions previously seen with solid Giemsa staining were re-examined with Q-banding and the identity of the chromosome involved was established

(Figure 4).

Approximately 10 spreads were further examined with fluorescence. Five to six good spreads, i.e., those with few or no overlaps, good banding resolution of stain and no interfering artifacts, were photographed for karyotyping.

d) Preparation of Karyotypes

Two karyotypes per patient were prepared from 8 X 10 inch metaphase enlargement prints. In the event of an abnormal finding, at least 3 karyotypes were used to substantiate the finding. Chromosome anomalies were further examined by other staining procedures.

6. Other Staining Procedures

a) G-banding (modification of Seabright, 1971)

A stock solution of trypsin (1:250 Difco Laboratories, Detroit, Michigan, U.S.A.) was prepared to a final concentration of 1.25 gm/25 ml distilled water and kept frozen. A working trypsin solution was prepared by adding 1 ml of the stock solution to 49 ml of 0.9% NaCl solution warmed to 37°C. The slides were placed in this trypsin solution for a predetermined length of time and then rinsed twice in 0.9% NaCl. The slides were then stained in 2% Giemsa (Gurr's R66) in Sörensen's phosphate buffer (pH 6.8) for 7 minutes, rinsed twice in distilled water and allowed to air dry.

b) C-banding (modification of Scheres, 1976)

Slides were incubated in a saturated solution of $Ba(OH)_2$ [2 gm $Ba(OH_2)$ in 100 ml distilled water] at 60-65°C.

for 6 minutes and rinsed with normal saline. The slides were then incubated in 2X SSC (standard sodium citrate) at 60-65°C for 40 min, rinsed with saline and stained in Giemsa (1 ml Harleco Giemsa in 39 ml pH=6.8 buffer) for 15-20 minutes. Slides were then rinsed in pH=6.8 buffer and air dried.

c) NOR-staining (Howell, 1981).

A stock colloidal developer solution (solution A) was prepared by adding 2 gm of powdered gelatin (Difco Bacto-gel) to 100 ml of deionized water containing 1 ml of pure formic acid. This solution was stirred for 10 minutes and stored for up to 2 weeks in a dark bottle. An aqueous 50% $AgNO_3$ solution (solution B) was prepared by adding 5 gm $AgNO_3$ to 10 ml of deionized water. This solution was stable.

The slides were aged for one week to enhance the banding quality. The precise mechanism(s) involved in this process is not known (ACT Laboratory Manual, 1980). The one week old slides were treated in the following manner. One drop of solution A was placed on each end of the slide. Two drops of solution B were then placed on each drop of solution A. The two solutions were mixed when a coverslip was wet-mounted. The slide was then placed on a hot plate pre-heated to 65-70°C. When the mixed solution turned golden brown (approximately 1 minute), the slide was rinsed with warm, deionized water to remove the coverslip and excess silver. The slide was then stained in 2% Giemsa

(Gurr's) for 15 seconds, rinsed once in distilled water and allowed to air dry.

d) Methyl green/DAPI C-bands (Donlon & Magenis, 1983)

A stock solution of methyl green (MG) was made by dissolving 1.76 gm of methyl green (Sigma Chemical Co.) into 100 ml of McIlvaines pH 4.0 buffer. A working solution (0.352 mg/ml) of MG was prepared just prior to staining by diluting 1 ml of the stock MG into 50 ml of McIlvaines pH 7.0 buffer.

A stock solution of 4',6-diamidino-2-phenyl-indole (DAPI) (Sigma Chemical Co.) was prepared by diluting 100 ug DAPI in 10 ml of deionized water. Two drops of methanol were added to help dissolve the DAPI. A working solution of DAPI was prepared by diluting 0.5 ml of stock in 50 ml of a 1:1 dilution of McIlvaines pH 7.0 buffer and deionized water. This solution can be stored at 4°C in a light-tight container.

One-day old slides were stained in a working solution of MG for 15-30 minutes and rinsed with buffer. Slides were then stained in DAPI for 5 minutes in the dark, returned to MG for an additional 1-2 minutes, and rinsed with buffer. The slides were mounted with two drops of a 1:1 dilution of glycerol and dilute McIlvaines pH 7.0 buffer and coverslipped. The slides were then examined on a Leitz photoscope (filter set #4).

e) R-banding (modification of Schested, 1974)

A Sorensen's buffer solution (pH 5.6) was heated to 86°C in a waterbath. Four to five day old air-dried slides were rinsed in cold tap water and incubated in the hot buffer solution for 20-30 minutes. Slides were agitated once every two minutes to remove air bubbles. The slides were then rinsed in distilled water, stained in Giemsa (2 ml Azure B in 48 ml distilled water) for 10 minutes, rinsed in distilled water and allowed to air dry.

f) Photomicroscopy

All photographs were taken using Kodak High Contrast Copy film (DIN 16, 35mm) and bright field microscopy with a green filter. A Planapo 63 X (Zeiss) objective and 1.6 OPTOVAR lens was used with immersion oil. Negatives were developed with Kodak D-19 for six minutes at 20°C. Photographs were printed on Kodak polycontrast rapid II RC paper. Chromosome analysis was done on 8 X 10 inch enlargement prints of complete metaphase spreads.

IV RESULTS

A. The Population Pool

In this study, the testicular volumes of 878 institutionalized, non-Down males were calculated. The mean volume and one standard deviation for each age was determined and plotted (Figure 6). Normal testicular development, as determined by various investigators, is summarized in Figure 7 and is compared to the rate of testicular enlargement in male retardates from the present survey. Testicular enlargement usually begins at approximately 12 years of age and terminates by 17 years (Figure 7). The testicular development curve in male retardates from the present study is shifted to the right of the normal curves, indicating delayed testicular enlargement among institutionalized males. Testicular enlargement in MR males from this survey begins at approximately 14-15 years and is not completed until 22 years.

Of the 878 residents examined, 817 were adult males (≥18 years of age). These adult males were segregated into three groups based on their testicular volume: a) macro-orchid ($V \geq 25$ ml), b) micro-orchid ($V \leq 15$ ml), c) normal ($15 < V < 25$ ml).

The distribution of testicular volume among institutionalized, non-Down syndrome, adult males was:

a) cryptorchid: 23 (2.8%)

b) macro-orchid: 170 (20.8%)

c) micro-orchid: 352 (43.1%)

d) normal: 272 (33.3%)

These results are summarized in Figure 8. The adult mean testicular volume was 19.5 ± 15.8 ml. The range was 0.6-177.0 ml.

B. Distribution of Testicular Volume According to Height

The overall mean height of adult institutionalized males was 164.1 ± 11.9 cm (Table 4). The adult mean height was also calculated for each volumetric group (Table 4). Micro-orchid males had a mean height of 161.2 ± 13.1 cm; macro-orchid males, 167.1 ± 11.7 cm; and males with normal testicular volume, 165.1 ± 9.3 cm. An analysis of variance showed a significant difference between these means ($F=9.39$, $\alpha = .05$) (Table 4). A multiple comparisons procedure, known as Tukey's test, was used to determine between which population means differences existed. The critical value of q was 164.669 at $\alpha = .05$. This value exceeded only the mean height of micro-orchid males. Therefore, micro-orchid males were significantly shorter than males with either macro-orchidism or normal testicular volume.

Correlation co-efficients for various physical features (testicular volume, height, and age) of adult (non-Down syndrome) mentally retarded males from the present study is shown in Table 5. Only two correlations were statistically significant. A positive correlation ($.01<p<.05$) was present

between height and micro-orchidism ($V \leq 15$ ml). A negative correlation ($p < .01$) was found between age and height in males with normal testicular volume ($15 < V < 25$ ml).

C. Distribution of Testicular Volume According to Diagnostic Groups in MR

All adult males with macro-orchidism (170) were cytogenetically examined. Of these, 5 cultures would not grow even after 5 attempts. Thus, karyotypes on a total of 165 macro-orchid adult males were obtained. Chromosome analyses were also conducted on 165 adult males with microorchidism and normal testicular volume who were selected by using a random numbers table. The medical records of all karyotypically normal residents were subsequently examined for other possible diagnoses related to MR (Table 6).

1. Chromosome Abnormalities

The total percentage of chromosome abnormalities in each volumetric group was: macro-orchid, 2.4%; micro-orchid, 4.2%; normal volume, 3.0% (Table 7). Apparently balanced translocations were found in 1.8% of macro-orchid males, 1.2% of micro-orchid males, and 0.6% of males with normal testicular volume. Other chromosome anomalies (deletions, trisomies, supernumaries) were seen in 0.6% of macro-orchid males, 3.0% of micro-orchid males, and 2.4% of males with normal testicular volume. These were not statistically

significant differences (Table 23). The stratified frequency (P_o) (see Appendix 2) of chromosome abnormalities was estimated to be 3.6% (Table 20).

Inv(3c) was found in 3.0% each of males with macro-orchidism and micro-orchidism and in 5.4% of residents with normal testicular volume (Table 7). These differences were again not statistically significant (Table 23).

A significant difference was observed in the occurrence of autosomal lesions (see section 34 in Materials and Methods) among the three volumetric groups (Table 7). Micro-orchid males were found to exhibit a significantly lower incidence of these lesions (2.4%) than either males with macro-orchidism (9.6%) (Z-test, two-sided, Z=2.635, α = 0.17) or normal testicular volume (9.0%) (Z-test, two-sided, Z=3.105, α = 0.17 (Table 23).

2. Non-specific XLMR

A highly significant difference was found in the distribution of the fra(X) syndrome among the three testicular volumetric groups (Table 8). Macro-orchid males had a much greater incidence of this disorder (10.3%) than males with either micro-orchidism (1.2%) or normal testicular volume (1.2%) (Z-test, two-sided, Z=3.600, α = .017) (Table 23). The P_o of the fra(X) syndrome among institutionalized, non-Down syndrome males was determined to be 3.1% (Table 20).

Fra(X) negative non-specific XLMR was postulated to be present in 49 probands. Of these, 18 were macro-orchid, 16 were micro-orchid and 15 were of normal volume (Table 8). These differences were not statistically significant (Table 23). The stratified frequency (P_s) of non-specific fra(X) negative XLMR was estimated to be 9.4% (Table 20).

When both fra(X) negative and positive non-specific XLMR were considered together (Table 8), a significantly greater percentage of macro-orchid males (21.2%) exhibited non-specific XLMR as compared to either males with micro-orchidism (10.9%) (Z-test, two sided, $Z=2.550$, $\alpha = .017$) or normal volume (10.3%) (Z-test, two-sided, $Z=2.700$, $\alpha=.017$) (Table 23). Therefore, although twice as many macro-orchid males had non-specific XLMR as males with either micro-orchidism or normal testicular volume, this difference was due to the statistically greater number of fra(X) positive as compared to fra(X) negative males (Table 8).

3. Other Genetic Factors Related to MR

Other genetic factors recorded as perhaps being causally related to MR included consanguinity, autosomal dominant and recessive disorders, syndromes of unknown etiology and non-specific familial MR (Table 9).

Consanguinity was identified in 1.8% of residents with macro-orchidism, in 2.4% of males with normal testicular volume, and in 1.2% of micro-orchid males. Two males with

macro-orchidism (1.2%) and three males with normal testicular volume (1.8%) were diagnosed as having tuberous sclerosis. The cranio-facial-digital syndrome was found in one resident with normal testicular volume (0.6%). One patient with micro-orchidism (0.6%) presented with neurofibromatosis.

Four (2.4%) patients with macro-orchidism were diagnosed as having phenylketonuria (PKU), compared to 3 (1.8%) with microorchidism and 1 (0.6%) with normal testicular volume. In addition, one resident with normal testicular volume was also postulated to have an undetermined metabolic disorder because of a strange odour in his urine. Other medical diagnoses as a cause for mental retardation included syndromes of unknown etiology such as the Sturge-Weber and de Lange syndromes.

A positive family history of MR not consistent with X-linkage was found in 50 pedigrees. The MR was of unspecified etiology and these pedigrees were therefore categorized as non-specific familial MR (Herbst and Miller, 1980). Such case histories were identified in 16 probands with macro-orchidism, 18 with micro-orchidism, and 16 with normal testicular volume (Table 9).

There were no significant differences in distribution among the three volumetric groups for any of the aforementioned disorders (Table 23). The overall frequences (P_o) of these disorders were (Table 20): consanguinity, 1.7%;

autosomal recessive, 1.7%; autosomal dominant, 1.3%; syndromes of unknown etiology, 0.5%; and non-specific familial MR, 10.4%.

4. Environmental Factors Related to MR

Specific external factors were also listed in the medical records of some residents as being causally related to MR (Table 10). These included:

1) prenatal infections and diseases such as toxoplasmosis, rubella, scarlet fever, rheumatic fever, and syphilis;
2) postnatal infections and diseases such as encephalitis, meningitis, high-fever, cerebral hemorrhage and brain tumour; and
3) other related factors such as status epilepticus, hypothyroidism, cranial calcifications, lead poisoning, meningeal adhesions, ABO incompatability, and MR following a major psychiatric disorder.

A significant difference in the incidence of environmentally-caused MR was found only for post-natal disease (Table 10). Macro-orchid males had a significantly lower frequency of post-natal disease (2.4%) than micro-orchid males (10.9%) (Z-test, two-sided, $Z=3.030$, $\alpha = .017$) (Table 23). Although a similar trend existed when macro-orchid males were compared to males with normal testicular volume, the Z value obtained was not statistically significant at the level of $\alpha = .017$ (.05/3) used to test the differences

between the three success probabilities, $\hat{p}_i$ (Table 23).

Trauma, in the form of birth injury, anoxia, and post-natal injury was also listed in the medical records as a possible contributing factor related to MR. None of these factors was recorded as the definitive cause of MR. There was no significant difference in distribution of these factors among the three volumetric groups (Table 23).

MR of unknown etiology was found in 43.6% of males with macro-orchidism, 41.8% of males with micro-orchidism, and 45.5% of males with normal volume (Table 10). These differences were not statistically significant (Table 23).

D. Neurological Disorders and Testicular Volume

A review of the medical records also revealed significant differences in the distribution of various neurological disorders such as cerebral palsy, spasticity, and paralysis (quadraplegia, paraplegia, or hemiplegia) (Table 11). Micro-orchid males had the highest incidence (24.2%) of spastic paralyses compared to either males with macro-orchidism (0%) (Z-test, two-sided, Z=12.306, α =.017) or normal volume (5.4%) (Z-test, two-sided, Z=4.793, α =.017). Micro-orchid males also had a higher frequency of paralysis (7.2%) (Table 11) than macro-orchid males (0.6%) (Z-test, two-sided, Z=3.175, α =.017) (Table 23). Although a similar trend existed when micro-orchid males were compared to males with normal volume, the calculated Z-score was not

significant at the level of $\alpha = .017$ (.05/3) used to test the differences among three $\hat{p}_i$'s (Table 23).

Epilepsy was present in 40.2% of macro-orchid males, 48.6% of micro-orchid males, and 42.6% of males with normal volume (Table 11). These differences were not statistically significant (Table 23).

E. Distribution of Cryptorchidism According to Diagnostic Groups in MR

Of the 23 males with cryptorchidism, 10 were examined cytogenetically. One patient was found to have a 47,XY, +inv dup(15)(p13→q12). None was found with the fra(X) and no autosomal lesions were observed. Examination of the case histories (Table 12) of these residents revealed 2 (20%) cases with non-specific familial MR. Two probands had MR due to environmental causes (rubella and encephalitis). The remainder had no specified cause of MR.

F. Analysis of Non-Specific XLMR

1. Family Pedigrees

The 49 individuals with fra(X) negative non-specific XLMR represented 42 families as some brothers and cousins from the families were selected for study before the pedigree information was available (Figures 9-11). They were residents of the same or different institutions.

In these families, after the removal of the probands and their mothers to eliminate ascertainment bias, there remained 63 affected (MR) males, 65 normal males, 18 affected females and 77 normal females. There was no significant deviation (Chi-square, $X^2 = 4.886$, $\alpha = 0.05$, df = 2) from an expected 2:1:1 ratio of females to normal males to affected males (Herbst and Miller,1980). If it is assumed that half of the total number (95) of females will be carriers, then 18 out of a possible 47.5 (37.9%) are intellectually handicapped.

Twenty-one male residents with the fra(X) syndrome were detected in this survey. These 21 individuals represent 16 apparently unrelated families as some brothers and cousins were found to reside in the same institution. The pedigrees are shown in Figures 12-27. Four out of sixteen (25%) families had only one sibship affected with MR with no other recorded history of MR (Figures 15-18). Nine out of sixteen (56%) families had a maternal family history of MR (Figures 19-27). There was no history of MR in the three remaining families (18.8%) (Figures 12-14) in which only the proband was found to be fra(X) positive.

The pedigree of family Fg (Figure 27) emphasizes the need for an accurate family and complete history in the study of MR. The initial proband R.Fg. (IV-142) was listed as abandoned and the only recorded next-of-kin was an aunt living in Sudbury, Ontario. The aunt was contacted by the

Public Health Clinic in Sudbury and was found to belong to the family Fg shown in Figure 27. This family was already being screened by the Public Health Clinic (Sudbury) for the fra(X) disorder. The sibship of probands V-17 and V-18 was at first considered as a separate family. However, it was discovered that these probands' mother (IV-96) was the sister-in-law of proband R.Fg.'s (IV-142) female first cousin (IV-94). This woman (IV-94) and her husband (IV-95) were found to be related. Although the exact nature of this relationship remains to be determined, they cannot be closer than being third cousins. Co-incidently, a woman (IV-70) was referred to our laboratory for fra(X) screening because of the presence of MR in her family. Family history information revealed that she and her immediate family were also related to fra(X) family Fg.

As was done for fra(X) negative non-specific XLMR, the segregation ratios expected for an X-linked disorder were applied to fra(X) pedigrees. These ratios were calculated by counting the number of affected and non-affected males versus the number of affected and non-affected females. As fra(X) screening could not be performed on all family members for a variety of reasons (refusal, lost contact, distance, etc.), the analysis was done using mental impairment as the marker for the fra(X) (Fishburn et al, 1983; Turner and Jacobs, 1984; Sherman et al, 1984a). It seems reasonable to assume that usually when two or more

close relatives are mentally affected, the etiology would be the same in each of those affected (Davison, 1973) unless other specific diagnoses are listed (e.g., Down syndrome) (Figure 19).

The probands and their mothers were subtracted from the total number to eliminate ascertainment bias. In addition, sibships with transmission from a "normal" male (II-3, Figure 25; I-7, Figure 26; I-1, Figure 27) were excluded. This left 37 normal males, 20 affected males, 48 normal females and 10 affected females. If half of the total number (58) of females are assumed to be carriers, then 10 out of a possible 29 (34.5%) are expressing heterozygotes. The ratio of females to normal males to affected males was 58:37:20. Although a deficit of affected males was noted, this difference was not statistically significant (Chi-square, $X^2=5.034$, $\alpha=0.05$, N=2) from the expected ratio of 2:1:1 (Herbst and Miller, 1980).

2. Physical Characteristics

a) Testicular Volume

Of the 32 males (21 probands and 12 relatives) found with the fra(X), 25 (21 probands and 4 relatives) consented to testicular measurement (Table 14, Figure 28). Eighteen of the 25 had a testicular volume $\geq$ 25 ml. A.Bl. (age 11 yr) (#1a, Figure 28) had a volume of 11 ml, well over the mean for his age (Figures 6 and 7). His brother, S.Bl.,

(age 20 yr) (#1b, Figure 28) had a volume of 53.2 ml. Thus 19/25 (76%) of males with the fra(X) exhibited macro-orchidism for their age group. The average testicular volume for fra(X) males was 49.1 ± 29.5 ml (Table 14).

Three of the 25 fra(X) males (12%) (D.D., J.H.W., and A.C.) had testicular volumes within the normal range (Figure 28). However, J.H.W. (#9) who had a testicular volume of 24.4 ml was still above the mean of 19.5 ml (Figure 8) for institutionalized males. A.C. (#3a) (V=17.1 ml) is the brother of W.C. (#3b) (V=42.3 ml) and T.C. (#3c) (V=63.3 ml). D.D. (#4a) (V=18.3 ml) is the first cousin of D.M. (#4b) (V=33.8 ml).

Three of the 25 fra(X) males (12%) with the fra(X) had a testicular volume ≤ 15 ml (D.B., B.O. and J.B.). D.B. (#2a) (V=11.8 ml) is the brother of W.B. (#2b) (V=44.9 ml) and half brother to R.W. (#2c) (V=33.1 ml).

The three probands with no family history of MR were all macro-orchid (J.K., D.R., H.L.) (Figure 28).

(b) Distribution of Testicular Volume in Non-Specific XLMR

The distribution of testicular volume in the 70 institutionalized males with non-specific XLMR [fra(X) positive: 21; fra(X) negative: 49] is shown in Figure 29. A distinct difference in distribution of these two disorders was observed at a volume of 40 ml.

Of the 21 fra(X) positive probands, 17(81.0%) had a testicular volume $\geq$ 25 ml, 2(9.5%) had testicular volume within the normal range and 2(9.5%) had a volume $\leq$ 15 ml. Of the 17 fra(X) positive males with a volume $\geq$ 25 ml, 13(61.9%) had a testicular volume $\geq$ 40 ml.

Of the 49 probands postulated to have fra(X) negative non-specific XLMR 18(36.7%) had a testicular volume $\geq$ 25 ml, 15(30.6%) had testicular volume within the normal range and 16(32.7%) had a volume $\leq$ 15 ml. Of the 18 fra(X) negative non-specific XLMR males with a volume $\geq$ 25 ml, only 1(2.1% had a testicular volume $\geq$ 40 ml.

(c) Height

The mean height of fra(X) males $\geq$ 18 yr was 169.3 $\pm$ 6.5 cm (Table 13). The adult mean heights of fra(X) negative males with non-specific XLMR are listed in Table 13 for comparison. An analysis of variance showed that there was no significant difference ($F=1.973$, $\alpha = .05$) in height among the four groups of males with XLMR. Fra(X) males were shown to be significantly taller than micro-orchid males in the general institutionalized male population (analysis of variance, $F = 8.06$, $\alpha = .05$; Tukey's test ($q = 165.4$, $\alpha = .05$) (Table 4).

The other physical characteristics of fra(X) males, as described in their clinical case histories, are summarized in Table 21.

3. Fra(X) Expression

(a) Percentage of Fra(X) Expression and Level of MR

The frequency of the fra(X) in affected males and carrier females is shown in Tables 15 and 16 respectively. The average percentage of fra(X) expression in affected males was 25.0%. There was no significant difference in the frequency of the fra(X) between different levels of MR (mild, moderate, severe and profound) (analysis of variance, $F=-2.015$, $\alpha = .05$). A more accurate correlation between fra(X) and IQ was not possible as very often only the different levels of MR were usually recorded in the case histories rather than actual IQ measurements. Although retarded female carriers demonstrated a higher percentage of fra(X) expression (13.0%) than non-retarded female carriers (10.0%), this difference was not statistically significant (student's t-test, $t=5.786$, $df=24$, $p < 0.05$).

A significant difference (student's t-test, $t=3.17$, $df=56$, $p < 0.001$) in expression was observed between fra(X) males (25.0%) and female carriers (10.6%).

(b) Relation to Age

The relationship between age and percentage of fra(X) expression in affected males is shown in Figure 30a. A linear regression analysis showed this relationship to be random ($t=.32$, $df=26$, $p < .01$).

A linear regression analysis between the percentage of fra(X) expression and age in affected (Figure 30b) ($t=1.105$, $df=4$, $p < .01$) and non-affected (Figure 30c) ($t=3.74$, $df=18$, $p < .01$) female carriers showed a decrease in fra(X) expression with increasing age. However, this relationship was statistically significant only for the unaffected female carriers (Figure 30c).

(c) Reproducibility of the Fra(X)

A positive diagnosis of fra(X) was normally made when this fragile site was observed in two consecutive cell cultures. This was done only to ensure that the fra(X) was not due to a random break. The fra(X) frequency in repeated cultures is summarized in Table 17. Because of the variability in quality of cell cultures due to various technical procedures including transport time, the highest fra(X) frequency observed was taken as the diagnostic percentage. A paired-sample t-test for correlation showed that there were no significant differences between cultures #1 and #2.

4. Ultrasound Analysis of Macro-testes

Ultrasound analysis (Dr. J. Pozsonyi, Department of Pediatrics, CPRI, personal communication) was performed on the testes of 25 male residents with macro-orchidism: 5 were fra(X) positive and 20 were fra(X) negative. No consistent, significant abnormalities were found. A small

amount of fluid was present in the tunica vaginalis bilaterally in some cases and a rather prominent ductal pattern was seen throughout both testes in others. The testes otherwise showed no definite internal abnormality. No mass lesion or other such anomaly was identified (Figure 67).

5. Endocrine Studies in the Fra(X) Syndrome

Plasma levels of testosterone, luteinizing hormone, and follicle stimulating hormone were performed on two fra(X) males with macro-orchidism (Dr. J. Pozsonyi, personal communication). The results were found to be within the normal range. Thyroid function was also found to be normal.

G. Other Chromosome Abnormalities

The chromosome anomalies detected in this survey are summarized in Table 18. A total number of 17 non-fra(X) chromosome abnormalities were found (Table 18). The estimated stratified frequency (P_o) for chromosome anomalies other than the fra(X) found in institutionalized non-Down syndrome males from this study was 3.6% (Table 20). There were three patients (P_o = 0.9%) with 46,XY,del(5)(p14→pter) and three (P_o = 0.8%) with 47,XY,+inv dup(15)(p13→q12) as determined by C-, NOR-, and MG/DAPI C-banding studies (Figures 62 and 63). There were six patients (P_o = 1.1%) with apparently balanced autosomal translocations. Three of these were found to be familial,

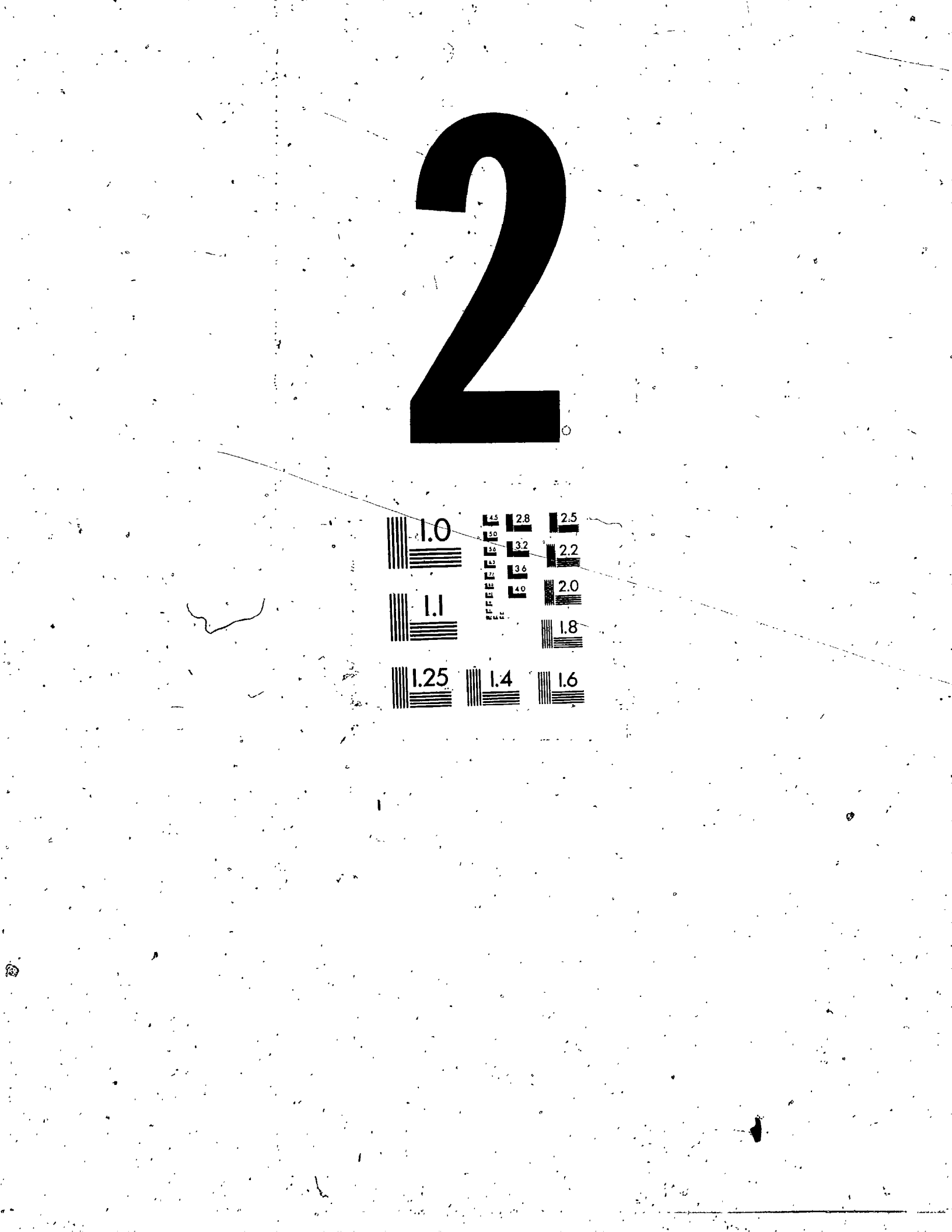
two were de novo, and no family information was available for one. Two sex chromosome abnormalities were found: one 47,XXY (P_o = 0.3%) and one 47,XYY (P_o= 0.1%). Other chromosome anomalies (P_o= 0.7%) that were found included a patient with a partial monosomy for the terminal portion of the short arm of chromosome #10 and two patients with partial trisomies. Of the two patients with partial trisomies, one had unidentified extra chromosome material on the terminal portion of the long arm of chromosome #8 and one had a duplication of band q22 on chromosome #21.

The karyotypes and pedigrees of patients with the aforementioned chromosome abnormalities are shown in Figures 32-66. Clinical features are described in Appendix 4.

Two patients with the karyotype, 46,XY,t(15;14) (q32;q13) were found to be brothers (Figure 45). A testicular biopsy was performed on the father of these two patients (Dr. F.R. Sergovich, CPRI, personal communication) (Figure 46). Column A is the actual meiotic configurations seen through the microscope. Column B is a diagrammatic illustration of the chromosomal structures seen in column A. Column C represents the interpretation of the associations involved between the translocated chromosomal segments.

H. Chromosome Polymorphisms

The incidences of inv(3c) in the macro-orchid, micro-orchid, and normal testicular volume groups were 3.0%, 3.0%


2
1.0
1.1
1.25
1.4
1.6
2.8
3.2
3.6
4.0
2.5
2.2
2.0
1.8

and 5.4% respectively (Table 7). There was no statistically significant difference among the three groups (Table 23). The stratified frequency (P_o) for the inv(3c) among institutionalized, non-Down syndrome males from the present study was estimated to be 3.7% (Table 20).

The incidence of inv(3c) was also examined in a non-retarded population. This population consisted of women $\geq$ 35 years who were referred to a genetic clinic for amniocentesis because of advanced maternal age. Examination of the amniocentesis records of Victoria Hospital (Dr. F.R. Sergovich, Cytogenetics Laboratory, Department of Pathology, Victoria Hospital, London, Ontario; personal communication) for mothers referred because of maternal age $\geq$ 35 years from 1974-1983 revealed a total frequency of 3.5% for 3(c) pericentric inversions (Table 19).

V Discussion

A. General

The present investigation is the first complete survey on the distribution of testicular volume among institutionalized (non-Down syndrome) males. This survey is one of the first studies to report a high incidence (20.8%) of macro-orchidism ($V \geq 25$ ml) among MR males (Pozsonyi et al, 1981). Previous studies on the incidence of abnormal testicular volume among MR males were limited to estimations on the occurence of hypogonadism. In these earlier studies, the presence of hypogonadism was determined by a number of variable parameters. These parameters included arbitrarily selected limits of micro-orchidism (Cassiman et al, 1975) and other physical features associated with hypogonadism (Nielsen and Fischer, 1965).

The present survey is unique in that it is the only study of MR males in which the incidences of cytogenetic disorders [fra(X) positive and negative non-specific XLMR and other chromosome anomalies] were determined by using testicular volume as a selection criterion. This approach was necessary in order to establish the clinical usefulness of abnormal testicular volume as a marker in the evaluation of cytogenetic disorders. The major findings from the present investigation are:

1) an incidence of 20.8% for macro-orchidism among institutionalized males (Pozsonyi et al, 1981); 43.1% for

micro-orchidism; 33.3% for normal testicular volume; and 2.8% for cryptorchidism;

2) the preferential association of macro-orchidism with the fra(X) syndrome;

3) the lack of correlation between testicular volume and fra(X) negative non-specific XLMR; and

4) the lack of correlation between micro-orchidism and the incidence of non-fra(X) chromosome abnormalities.

A testicular volume of 25 ml is generally defined as the 95th percentile in normal adult male populations (Zachmann et al, 1974). The etiology of the increased incidence of macro-orchidism among MR males is not known. Histological studies of macro-orchid testes have shown no consistent pathological changes (Ruvalcaba et al, 1977; Cantu et al, 1978). Ultrasound studies of macrotestes in the present survey (Dr. J. Pozsonyi, personal communication) also revealed no consistent anatomical abnormalities (Figure 67).

Non-specific XLMR was found to be present in 21.2% of macro-orchidic MR males. One-half of these males (10.3%) were fra(X) positive. The incidence of either micro-orchidism or normal testicular volume in males with the fra(X) syndrome was 1.2%. Macro-orchidism thus appears to be preferentially associated with the fra(X) syndrome. The incidence of macro-orchidism (10.9%), micro-orchidism (9.1%) and normal testicular volume (9.7%) among fra(X)

negative males with postulated non-specific XLMR was determined to be approximately equal. Thus, there is no preferential distribution of testicular volume among males with fra(X) negative non-specific XLMR.

The present study on the association between macro-orchidism and the fra(X) syndrome further established the use of a testicular volume $\geq$ 25 ml as a clinical marker in the diagnosis of the fra(X) syndrome. It was observed that fra(X) positive males had larger testes ($\geq$ 40 ml) than fra(X) negative males with non-specific XLMR. This finding suggests that testes exceeding 40 ml are a feature specific of the fra(X) syndrome and not just examples of general macro-orchidism associated with non-specific XLMR. The intra-familial variation of testicular volume observed in fra(X) families may result from variable expressivity of the fra(X) 'gene'. This suggestion is supported by the finding of a family history of macro-orchidism in fra(X) positive males who have micro-orchidism or normal testicular volume. Fra(X) negative non-specific XLMR with associated micro-orchidism or normal testicular volume may be representative of the Renpenning form of non-specific XLMR which is characterized by small testes, microcephaly, and short stature (Renpenning et al, 1962; Fox et al, 1980).

The use of testicular volume as a clinical marker in the diagnosis of non-fra(X) chromosome abnormalities is of little value. Although a larger number of chromosome

anomalies detected in this survey were present in MR males with micro-orchidism or cryptorchidism, the distribution of these abnormalities in the three volumetric categories did not differ from random expectation. Therefore, micro-orchidism is not a useful clinical marker for the evaluation of individuals with possible non-Down chromosome abnormalities.

B. Testicular Volume in Institutionalized Males

The mean testicular volume in institutionalized, non-Down males from the present study was calculated to 19.5 ± 15.8 ml by using caliper measurements (Figure 8). This value is comparable to reported normal adult means of 17-19 ml (Laron and Zilka, 1969; Farkas, 1970; Zachmann et al, 1974; Boisen, 1979). Rundle and Sylvester (1962) reported a mean adult volume of 31.1 ± 9.5 ml for mentally retarded males. However, their calculations were based on the formula of Lambert (1951) rather than Cantu (1976) (see section D2 in Literature Review) and it was generally accepted that this resulted in volume determinations which would exceed other reported values (Figure 7).

1. Testicular Volume and Height

Normal adult males have an average height of 174.7 cm (Partington, 1984), the 50th percentile being 177 cm (NCHS, 1976). Institutionalized adult males are reported to be at least 10 cm shorter than their normal peers (Mosier et al,

1965). Adult male residents from this study have an overall mean height of 164.1 ± 11.9 cm, which is in agreement with that reported by Mosier et al (1965). This value is below the 5th percentile of normal adult male height (165 cm) (NCHS, 1976).

Determinations of adult mean heights for each of the three testicular volume groups demonstrated that micro-orchid males were significantly shorter (161.2 ± 13.1 cm) than males with either macro-orchidism (167.1 ± 11.7 cm) or normal volume (165.2 ± 9.3) (Table 5). A small but significant correlation ($0.01 < p < 0.05$) was found between height and testicular volumes less than 15 ml (Table 5). No correlation was present between height and volumes greater than 15 ml (Table 5). Sylvester and Rundle (1962) and Padron et al (1979) also found no correlation between height and testicular volume in normal adult males. A small correlation was observed between height and volume in normal adolescent males (Rundle and Sylvester, 1962). The present observations together with those of Rundle and Sylvester (1962) suggest that small testicular size ($V \leq 15$ ml) is influenced to some extent by general body size and that enlarged testes ($V \geq 25$ ml) are not accompanied by a concomittant increase in body size. This latter finding is in agreement with Boisen (1979) who reported that chromosomally normal "tall" (≥ 184 cm) males had normal testicular volume (17.5 ml).

No correlation was found between age and testicular

volume (Table 5). This finding is in contrast to the study of Baker et al (1975) in which a correlation between age and testicular volume was observed. These authors reported that testicular size decreased with increasing age. However, their study consisted of a large number of geriatric patients whose ages were > 70 yr. The observed decrease in testicular volume is probably the result of testicular atrophy (Baker et al, 1975).

A significant negative correlation ($p < .01$) between age and height was found only in MR males with normal testicular volume ($15<V<25$ ml) (Table 5). This finding indicates a decrease in height with increasing age in males with normal testicular volume. A slight decrease in height is also found in normal men aged $\geq$ 60 years and is attributed to osteoporotic changes in the bone (Suitor and Hunter, 1980). A pronounced age effect in the MR males with normal testicular volume from the present study may occur as a result of their growth retardation. The reason for the lack of correlation between age and height in micro-orchid and macro-orchid males is not known.

2. Testicular Development

Testicular development in mentally retarded males from the present study was delayed as compared to normal male populations (Figure 7). The testes normally begin to enlarge at approximately 12 yr and reach adult size by 17 yr (Marshall and Tanner, 1970) (Figure 7). Testicular growth

in institutionalized males from this study did not appear to begin until 14-15 yr (Figure 7). The developmental period was prolonged and did not plateau until 22 yr (Figure 7).

This finding is in contrast to that of Rundle and Sylvester (1962) who reported that testicular enlargement in institutionalized males took place normally between the ages 12-17 yr. Their study consisted of 260 institutionalized, non-Down males. None of these MR males had what were referred to as "spastic deformities" (Rundle and Sylvester, 1962). Although the precise nature of these "spastic deformities" was not stated, they presumably referred to cerebral palsy and/or spastic paralyses. The overall incidence (P_o) of these neurological disorders among institutionalized males from the present study was 23.7% (Table 20). The high incidence of such disorders in this survey as compared to 0% in the study of Rundle and Sylvester (1962) suggests that the presence of neuropathological diseases may delay the rate of testicular development observed in this study.

Indirect evidence to support this suggestion comes from the finding in the present study that a significant difference [$t_{(2)} = 6.32$, df = 490, $\alpha = .001$] was present between the adult mean testicular volumes of MR non-Down males with (12.8 $\pm$ 7.1 ml) and without (26.0 $\pm$ 20.6 ml) neurological disorders. The mean testicular volume of MR males without neurological disorders (26.0 $\pm$ 20.6 ml) exceeds the normal adult mean of 17-19 ml (Laron and Zilka, 1969; Farkas, 1976;

Zachman et al, 1974; Boisen, 1979). The larger testicular volumes reported by Rundle and Sylvester (1962) can thus result not only from the different formula used to calculate volume, but also from the absence of MR males with "spastic deformities" in their study. Since testicular development is indicated by testicular volume, the apparent delay in testicular growth in the present study may be a result of the presence of MR males with neurological damage whose testicular volumes were found to be reduced.

Micro-orchid males from the present study were found to have a significantly higher incidence of spastic/paralytic disorders than males with either macro-orchidism or normal testicular volume (Table 11). Paraplegia resulting from spinal cord trauma is a known cause of hypogonadism (Reichlin, 1974). The majority of cases of hemiplegia also appear to be the result of brain damage sustained during the prenatal period (Ingram, 1966). Tizard (1977) reported an association between spastic diplegia, hemiplegia, and tetraplegia with pre-term delivery and fetal/interpartum asphyxia. The significantly greater frequency of spastic disorders in micro-orchid males (Table 11) suggests that a large portion of the MR found in these males may be the result of prenatal or birth injury. (Approximately 71% of micro-orchid males with spastic/paralytic disorders had MR of unspecified etiology.) The resulting neuropathological conditions could cause hypogonadism which is manifested as a decrease in

testicular size. This suggestion is supported by the finding that macro-orchid males have the lowest incidence of spastic paralyses of the three volumetric groups (Table 11).

3. Distribution of Testicular Volume

Macro-orchidism ($V \geq 25$ ml) was found to be present in 20.8% of institutionalized males from this study (Pozsonyi et al, 1981). This finding is substantiated by Howard-Peebles and Finley (1983) and Brondum-Nielsen et al (1982) who reported that the incidence of macro-orchidism among MR males was 20% and 29% respectively. Howard-Peebles and Finley (1983) found testes greater than 34 ml in 8.3% of retarded males. This incidence is in agreement with the present finding that 8.1% of residents had volumes exceeding 35 ml (Figure 8).

Testicular volumes of $\leq$ 10 ml and $\leq$ 5 ml were found in 20% and 2.7% respectively of adult residents from this study. Bilateral cryptorchidism was present in 2.8% of males. These results are in contrast to the report of Cassiman et al (1975) that only 1.9% of adult MR males had hypogonadism: 1.5% had a testicular volume $\leq$ 8.32 ml (see section D3a in Literature Review) and 0.4% had bilateral cryptorchidism. The incidence of bilateral cryptorchidism among normal males is reported to be approximately 0.1% (Paulsen, 1974).

The high incidence of micro-orchidism/cryptorchidism found in the present study may be explained by the

composition of the sample populations. The present investigation excluded Down syndrome males whereas the survey of Cassiman et al (1975) included all male residents. However, Sylvester and Rundle (1962) found that although testicular size was reduced in adult Down syndrome males, the frequency of cryptorchidism in these males was not higher than that in non-Down MR patients. Thus, the high frequency of microorchidism/cryptorchidism found in the present study does not appear to result from exclusion of Down syndrome patients.

The low frequency of hypogonadism reported by Cassiman et al (1975) may be a result of their method of determining testicular size. Cassiman et al (1975) initially screened adult MR males "by rapid examination" for the presence of hypogonadism. The precise method of volume determination was not stated. The testes of males selected in this manner were subsequently measured with calipers. Therefore, it is possible that the number of hypogonadic males may have been underestimated in Cassiman's et al (1975) initial screening.

The distribution of testicular volume among adult (non-Down syndrome) MR males in the present study is skewed at the upper ($V \geq 25$ ml) and lower ($V \leq 10$ ml) ends of the normal distribution curve. As previously stated, a testicular volume of 25 ml is defined as the 95th percentile in normal adult males (Zachmann et al, 1974). Testicular

volumes $\geq$ 25 ml were found in 20.8% of MR males from the present study, a fourfold increase over normal distribution. A testicular volume of 10 ml is defined as the 5th percentile in normal adult males (Zachmann et al, 1974). Volumes $\leq$ 10 ml were found in 20% of males from the present study. This latter value is also a fourfold increase over normal distribution.

C. Distribution of Testicular Volume According to MR Diagnostic Groups

1. Chromosome Abnormalities

The overall frequency (P_o) of chromosome abnormalities, excluding the fra(X), in the present study was 3.6% (Table 20). This is comparable to the frequency of 3.0% found in other institutionalized surveys if Down syndrome patients are also excluded (Table 2). Although more micro-orchid patients (4.2%) had non-fra(X) chromosome abnormalities than macro-orchid patients (2.4%) or patients with normal volume (3.0%), this difference was not statistically different (Table 7).

Balanced translocations did not appear to be associated preferentially with micro-orchidism in this study (Table 7). Previous reports (Chandley et al, 1976; Handelsman and Smith, 1983) have shown that balanced reciprocal autosomal translocations can cause oligospermia or azoospermia, resulting in hypogonadism and reduced testicular size (Chandley et al, 1976). In the present study, only 2 of the 6 males

with apparently balanced translocations had a testicular volume of less than 15 ml (T.W. and J.T., Table 18). In addition, the brother of T.W. (R.W., Table 18) had the same translocation and he was macro-orchidic. The effect of balanced autosomal translocations on testicular function thus appears to be variable.

No significant differences were found in the incidence of the chromosome heteromorphism, inv(3c) (as determined by Q banding), in the three volume groups from this study. The overall frequency (P_o) of inv(3c) among adult MR males in the present study was 3.7% (Table 20) as compared to an incidence of 3.5% in a non-retarded adult, female population (Table 19). These values are within the range described in other studies (Lin et al, 1976; Soudek and Sroka, 1979; Mikelsaar et al, 1978). The frequencies of inv(3c) found in the retarded and non-retarded populations from the present study support previous reports that there is no preferential association between the inv(3c) and MR (Soudek and Sroka, 1979; Mikelsaar et al, 1978).

Micro-orchidic residents had a significantly lower frequency of autosomal lesions (2.4%) than residents with normal testicular volume (9.0%) or macro-orchidism (9.6%) (Table 7). It can thus be postulated that genetic mechanisms involved in the formation of folic acid dependant fragile sites or autosomal lesions play a lesser role in micro-orchid residents. The reason(s) for this lesser role

in micro-orchid residents is (are) not known.

2. Non-specific XLMR

a) Fra(X) positive

A significantly higher proportion (10.3%) of macro-orchid males showed the fra(X) as compared to 1.2% each of males with micro-orchidism or normal testicular volume (Table 8). From the 15 fra(X) families found in this survey, the testicular volumes of 25 fra(X) positive males (21 probands and 4 relatives) were calculated. Of these 25 fra(X) males, 19 (76%) had a volume $\geq$ 25 ml, at least one standard deviation above the mean for their age group (Table 14 and Figure 6). This is in agreement with the results of Partington (1984) who found that 80% of fra(X) males had a testicular volume $\geq$ 30 ml.

Contrary to the report by Partington (1984) that macro-orchidism could not be found in pre-pubertal males, the present study showed that an 11-year-old fra(X) male, A.B. (Figure 28, #1a) had a testicular volume of 11.3. This value was well above the mean of 1.7 ml for pre-pubertal males (Laron and Zilka, 1969). Recently, Jenkins et al (1984) also reported the presence of macro-orchidism as the only apparent phenotypic abnormality in two fra(X) male fetuses.

The present study has confirmed the preferential association of macro-orchidism with the fra(X) syndrome.

However, enlarged testes are not specific to the fra(X) disorder as macro-orchidism also occurs in fra(X) negative non-specific XLMR (see following section).

It has been suggested by Partington (1984) and Meryash et al (1984) that the yield of fra(X) screening study would be improved if one were to examine cytogenetically only those males whose average testicular volume was at least 30 or 32 ml respectively. In the present study, the distribution of testicular volume in probands with nonspecific XLMR (Figure 29) showed that a testicular volume $\geq$ 40 ml can be a more appropriate clinical marker. Only 2.1% of probands with postulated non-specific XLMR without the fra(X) had volumes $\geq$ 40 as compared to 61.9% of fra(X) index cases. Testicular volumes $\geq$ 30 ml were found in 27% of probands with fra(X) negative non-specific XLMR as compared to 76% of fra(X) index cases. It thus appears that for a "high yield" of fra(X) cases, a testicular volume of 40 ml should be used as the lower limit.

Meryash et al (1984) has further suggested de-emphasizing macro-orchidism as a characteristic of the fra(X) disorder as it is "impractical" for non-medical professionals to examine mentally retarded males for large testes. This is felt to be an unjustified statement. Such patients are almost invariably referred to cytogenetic laboratories by physicians who can easily measure testicular volume as part of a routine physical examination. Similarly,

residents in institutions usually have routine annual physical examinations at which time a determination of testicular size could be easily accomplished.

Of the two fra(X) probands who had normal testicular volume (A.C. and D.D.) in the present study both had mentally retarded macro-orchid brothers (Figure 28). The two fra(X) probands with micro-orchidism (J.B. and B.O.) had mentally retarded male relatives whose testicular measurements were not available. J.B. was quite elderly (67 years) and confined to a wheelchair. It is possible that his micro-orchidism was the result of testicular atrophy. B.O. had associated spasticity (Table 21), which may have been related to his reduced testicular volume. Of the three fra(X) males with an apparently negative family history of MR (Figure 28), all had a testicular volume exceeding 40 ml.

Therefore, in screening for the fra(X) disorder, a family history of macro-orchidism (especially volumes exceeding 40 ml) and MR should be considered together as being useful clinical markers. The necessity to obtain accurate testicular measurements in families with possible non-specific XLMR should be emphasized. A testicular volume greater than 40 ml should serve as an immediate indication for fra(X) analysis. The further examination of those males who have lesser degrees of testicular enlargement (volumes less then 40 ml) should be determined following a review of family history and other clinical findings. Using these

criteria, all of the fra(X) males from the present survey would have been detected.

b) Fra(X) negative

Fra(X) negative non-specific XLMR was postulated to be present in 49 probands from the present study (Table 8). There was no preferential distribution of this disorder in the three testicular volumetric groups (Table 23). The overall frequency (P_o) for fra(X) negative non-specific XLMR found in the present study was 9.4% (Table 20). These 49 probands were found to belong to 42 different families (Figure 9-11).

The incidence of fra(X) negative non-specific XLMR is difficult to determine because of the inability to distinguish clinically this type of MR from non-specific MR that is not due to recessive X-linked genes. Apart from the fra(X) syndrome, a pattern of X-linked MR in a pedigree is, at present, the only means of identifying non-specific XLMR (Herbst and Miller, 1980). On the assumption that the pedigrees shown in Figures 9-11 are representative of fra(X) negative non-specific XLMR, a P_o of 9.4% (Table 20) for this disorder may be a minimum esimation since it is impossible to detect de novo cases or cases which do not follow a pattern of X-linkage. It can be demonstrated that of the 16 fra(X) families identified, 4 (25%) would not have been categorized as XLMR according to the definitions outlined in section A1 in Materials and Methods. In these 4

families, 3 probands (Figures 12-14) were possible de novo cases (the proband was the only affected individual) and the remaining proband (Figure 24) had an affected female relative (a maternal first cousin).

If it can be assumed that the findings of fra(X) families apply equally to those that are fra(X) negative, then the 42 pedigrees with postulated fra(X) negative non-specific XLMR may represent only 75% of the total because only cases in which family members show a suggestive pattern of X-linked transmission can be identified. The cases that cannot be detected might result from any of the following: the lack of family data; the traceable family is too small; a recent mutation which does not show an X-linkage; incomplete recessivity in females; and lack of expression in males.

In the present study, thirteen families with no maternal history of MR were postulated to represent fra(X) negative non-specific XLMR based on the presence of multiple MR males in the proband's sibship (Figure 9: #5, 6, 11, 13, 14, 15; Figure 10: #27, 32; Figure 11: 33, 34, 35, 37, 40). A more stringent definition for X-linkage also requires that a maternal history be present. Therefore, the distribution of fra(X) negative non-specific XLMR among the three testicular volume groups was re-examined

excluding the aforementioned pedigrees (Appendix 3). No significant difference in distribution was observed among the three groups (Appendix 3). Likewise, the ratio of females:normal males:affected males was 78:47:49 and did not differ significantly (Chi-square, $X=1.91$, $N=2$, $\alpha=.05$) from the expected ratio of 2:1:1 (Herbst and Miller, 1980).

c) Non-specific XLMR and Testicular Volume

Non-specific XLMR, both fra(X) positive and negative, was detected twice as often in macro-orchid males (21.2%) as in males with micro-orchidism (10.9%) or normal testicular volume (10.3%) (Table 8). This difference was the result of the high incidence of the fra(X) syndrome in macro-orchid patients (Table 8). Approximately one-half (17/35) of the males with non-specific XLMR and macro-orchidism exhibited the fra(X) as compared to 2/18 with micro-orchidism and 2/17 males with normal volume (Table 8).

It is not known whether fra(X) positive and negative XLMR patients belong to the same clinicopathological entity (Turner and Jacobs, 1984). Turner and Opitz (1980) and Fishburn et al (1983) have subdivided non-specific XLMR with macro-orchidism into fra(X) positive and negative

subgroups. The only evidence in support of this hypothesis was provided in a biochemical study by Langenbeck et al (1984). It was shown that fra(X) positive patients with macro-orchidism had higher levels of mean corpuscular haemoglobin (MCH) than non-retarded male controls or fra(X) negative patients with MR and macro-orchidism.

Fra(X) negative probands with micro-orchidism and normal testicular volume in the present study may be representative of the Renpenning form of non-specific XLMR, which is characterized by small to normal size testes, reduced stature, and microcephaly (Renpenning et al, 1962; Fox et al, 1980).

An inconsistency of testicular volume among affected male relatives from families with fra(X) negative (Figure 9: #10; Figure 10: #3, 4, 11, 25) and fra(X) positive (Figure 28) non-specific XLMR has been observed. This inconsistency may occur as a result of variable expressitivity of the X-linked gene.

The intra-familial variation in testicular size indicates that a family history of macro-orchidism should serve as a clinical marker in the diagnosis of the fra(X) syndrome.

3. Other Genetic Factors Related to MR

a) Consanguinity

The incidence of consanguinity in the general population is influenced by the type of population examined (urban or rural) as well as a number of religious, geographic and ethnic factors (Vogel and Motulsky, 1979). The incidence of consanguinity in the general population can thus be variable but is estimated generally to be 0.5-1.0% in industrialized areas (Swift et al, 1973; Vogel and Motulsky, 1979).

It has long been recognized that the frequency of consanguinity is higher among mentally retarded individuals than in the general population (Swift et al, 1973; Penrose, 1963). The most plausible explanation for finding an elevated frequency of consanguinity among institutionalized individuals is that these patients could be homozygous for harmful recessive genes whose expression is not presently recognizable either clinically or biochemically (Swift et al, 1973).

In the present survey, the overall frequency (P_o) of MR males postulated to be the result of consanguinity was 1.7% (Table 20). There was no significant deviation in the distribution of the incidence of consanguinity beween the three volumetric groups (Table 9). Other studies on the presence of consanguinity among parents of MR individuals recorded incidences of 2.4-8.6% (Mounoud et al, 1976; Klein et al, 1982; Sinclair, 1972; Swift et al, 1973).

b) Defined Syndromes

Some malformation syndromes associated with MR are known to result from single mutant genes (autosomal dominant, autosomal recessive, and X-linked) (Milunsky, 1975). In the present survey, MR syndromes resulting from autosomal dominant (tuberous sclerosis, neurofibromatosis, craniofacial dyostosis) and autosomal recessive (PKU) mutant genes were detected. No specific X-linked disorders were found. Other known causes for MR detected in the present survey included recognizable syndromes of unknown etiology such as the Sturge-Weber and deLange syndromes.

The overall frequency (P_o) of the aforementioned disorders (autosomal dominant, autosomal recessive, unknown etiology) in the present study was 3.5% (Table 20). There was no significant difference in the total distribution of these conditions among the three testicular volumetric groups.

c) Non-specific familial MR

A positive family history of MR which did not conform to X-linkage (as defined in section A1 in Materials and Methods) was found at an overall frequency (P_o) of 10.4% in institutionalized males from the present study (Table 20). In the absence of apparent X-linkage, the only possible classification for this category is non-specific familial MR (Herbst and Miller, 1980). However, based on the earlier finding that 25% of fra(X) families did not show

a pattern of inheritance consistent with X-linkage, possible X-linkage in some cases of non-specific familial MR should not be discounted. It is also possible that some of the cases of non-specific familial MR could have a minute chromosome abnormality undetectable by present cytogenetic techniques (Opitz et al, 1978).

There was no significant difference in the incidence of non-specific familial MR among the three testicular volume groups (Table 9).

4. Environmental Causes

The overall frequency (P_o) of environmentally caused MR was 24.3% (Table 20). Previously reported institutional estimates have ranged from 13-35% (Moser and Wolf, 1971: McDonald, 1973; Mounoud et al, 1976; Opitz et al, 1978; Opitz, 1979; Cortada and Kousseff, 1984).

Macro-orchid males were found to have a significantly lower incidence of post-natal infections as compared to micro-orchid males (Table 10). Infections such as encephalitis are known to be a cause of hypogonadism (Reichlin, 1974). The lower incidence of such infections among macro-orchid males indirectly supports the previous suggestion (see section B2 in Discussion) that decreased testicular size in institutionalized males may be associated with neuropathological mechanisms.

5. Unspecified Etiology

No specific cause for MR could be determined in 43.7% (P_o) (Table 20) of institutionalized males from this study. Family histories revealed a negative history of MR. Cytogenetic analysis failed to reveal chromosome abnormalities. Many residents in this category had various congenital malformations of the skull, face, ears, palate, and limbs or various parts of the body. These anomalies did not fit into clinically defined syndromes or patterns.

The observed MR in this group of institutionalized males could result from any of the following disorders (Opitz et al, 1978): 1) provisionally private syndromes resulting from de novo autosomal recessive Mendelian mutations; 2) minute chromosome abnormalities; 3) sporadic idiopathic multiple congenital anomalies (MCA); and 4) primary central nervous system (CNS) developmental defects such as microcephaly, hydrocephaly, and spina bifida (excluding known MCA syndromes with CNS malformations). Opitz et al (1978) also included cerebral palsy/seizures/hypotonia as a diagnostic category significantly correlated to MR if no other specifically identifiable causes were found.

Epilepsy associated with MR has been one of the criteria previously used to eliminate probands from familial studies (Penrose, 1938; Reed and Reed, 1965; Opitz, 1979; Opitz et al, 1978). It was thought that frequent seizures could cause or contribute to MR. Since epilepsy is heterogenous

in origin (Davison et al, 1973), the number of families with XLMR in a study may be artificially reduced by elimination of such probands (Herbst, 1980). For these reasons, no such correlation was attempted in the present study. It is interesting to note, however, that 44.3% (Table 20) of probands were epileptic. Previously reported frequencies ranged from 10-50% (Penrose, 1938; Opitz, 1979; Opitz et al, 1978; Laxova et al, 1977; Cortada and Kousseff, 1984). There was no significant difference in distribution beween the three testicular volume groups (Table 11).

D. Cytogenetic Characterizations

1. Non-specific XLMR

a) Frequency

A total of 70 males [49 fra(X) negative and 21 fra(X) positive] were found with non-specific XLMR in the present survey. This gave an overall frequency (P_o) of 12.4% (Table 20) for non-specific XLMR among institutionalized, non-Down syndrome males. The incidence of Down syndrome among males in the institutions which participated in this study was also 12.4% (Table 22). Non-specific XLMR thus appears to be the second most common genetic cause of MR among institutionalized males, with Down syndrome being the most common cause.

The 70 individuals identified in this survey with non-specific XLMR represent 58 unrelated families. Of these

58 families, 16 (28%) are due to the fra(X). This is in agreement with previous studies which have suggested that approximately one-third (Turner et al, 1980a) to one-half (Herbst and Miller, 1980) of all cases of non-specific XLMR are associated with the fra(X).

The fra(X) syndrome was the most frequently identified chromosome anomaly in this study. The overall frequency (P_o) of the fra(X) syndrome among institutionalized males from the present survey was 3.1% (Table 20). The P_o of other (non-Down) chromosome abnormalities ranged from 0.1-1.1% (Table 20) with a total P_o for these anomalies of 3.6% (Table 20). These findings suggest that, following Down syndrome, the fra(X) syndrome is the second most commonly diagnosed chromosomal disorder among MR males (Table 20).

b) Physical Characteristics

The clinical features of the fra(X) males detected in the present study are summarized in Table 21.

i) Macro-orchidism

The preferential association of macro-orchidism with the fra(X) syndrome has been previously described (see section C2a in Discussion). Ultrasound analysis of macro-orchid testes from both fra(X) positive and negative males revealed no consistent pathological abnormality (Figure 67) (Dr. J. Pozsonyi, personal communication). These results are in agreement with those of other

investigators (Turner et al, 1975; Ruvacalba et al, 1977; Cantu et al, 1978; Bowen et al, 1978) who also could not find consistent histological changes in macrotestes. Jenkins et al (1984) reported that enlarged interstitial lymphatics were the only testicular anomaly found in two macro-orchid fra(X) male fetuses. It was suggested that a lymphangiectatic mechanism was responsible for macroorchidism.

ii) Height

Partington (1984) found that fra(X) males were shorter (171.4 cm) than normal adult males (174.7 cm). This finding was confirmed in another study by Meryash et al (1984). Furthermore, Meryash et al (1984) suggested that institutionalization did not account for the reduced stature.

The mean adult height of the fra(X) positive males from the present study was 169.3 $\pm$ 6.5 cm which was approximately 5 cm below that of normal males. This finding is in agreement with the findings of Partington (1984) and Meryash et al (1984). Partington (1984) also suggested that reported observations of increased adult stature in fra(X) males might have resulted from comparisons of fra(X) with the generally shorter population of MR males (Mosier et al, 1965). This suggestion has been substantiated by the present study. Institutionalized adult males in this survey had an overall mean height of 164.1 $\pm$ 11.9 cm, as compared to a mean height of 169.3 $\pm$ 6.5 cm for fra(X) males. This difference in height would give an impression of increased

stature for the fra(X) males. However, when the adult mean height of fra(X) males was compared to the adult mean heights of MR males in each testicular volume group (Table 4), fra(X) males were found to be significantly taller than micro-orchid males. No significant difference in height was found between fra(X) males and MR males with macro-orchidism or normal testicular volume. In addition, no significant difference was found between the heights of fra(X) positive males and fra(X) negative probands with postulated non-specific XLMR (Table 13). Thus, excluding micro-orchid males, fra(X) males were not significantly taller than other male retardates, with or without XLMR.

c) Autism and the fra(X)

It has been reported by other investigators that there is a high incidence of autism among fra(X) patients (Turner et al, 1980a; Proops and Webb, 1981; Brown et al, 1982; Meryash et al, 1982; Watson et al, 1984). In the present study, no definite association was found between autism and the fra(X). Only 1/26 fra(X) males had a definite diagnosis of autism (Table 21). In another two cases, the diagnosis was questionnable. This finding is in agreement with that of Venter et al (1984) who reported no cases of fra(X) among 57 autistic children. These 57 children were selected randomly and not on the basis of a positive family history of either MR or autism. This method of selection is different from the method used by Brown et al (1982), in which

selection was based on a positive history of MR.

In an attempt to clarify the association between autism and the fra(X), an additional 15 autistic males (courtesy of Dr. S. Fisman, CPRI) were examined cytogenetically by the author for the fra(X). These males were not part of the institutional survey of this study and were randomly selected. None of the 15 males studied showed the fra(X). This finding was in agreement with that of Venter et al (1984). It has been suggested that the association, if any, between autism and the fra(X) could be better resolved through a large scale comparative study of unselected autistic patients and those selected on the basis of a positive family history for MR and/or autism (Venter et al, 1984).

d) Segregation Analysis

i) Hemizygote Expression

Segregation analysis of fra(X) pedigrees from the present study showed a ratio of 58:37:20 for females to normal males to affected males. Although a deficit of affected males was found, this ratio did not differ significantly from the expected 2:1:1 ratio (Herbst and Miller, 1980).

A survey of published fra(X) pedigrees* showed that

*See references 1, 3, 6, 7, 8, 12, 14, 16-18, 21-23, 24-27, 28, 30, 31-35, 37, 39-42; pp. 134-135.

there were 272 normal males, 193 affected males, 396 normal females and 83 affected females. These numbers were determined following removal of the probands and their mothers, and exclusion of the sibship with possible male transmission in order to eliminate ascertainment bias. The resulting ratio of females to normal males to affected males (479:272:193) differs significantly from the expected ratio of 2:1:1 (Chi-square, $X^2 = 13.42$, N=2, $\alpha = .05$) (Herbst and Miller, 1980). A deficit of affected males was also indicated from this literature survey. The results of this survey are in agreement with those of Sherman et al (1984a), who found a 20% deficit of affected males in an analysis of 110 fra(X) pedigrees. This deficit of affected males has been postulated to result from incomplete penetrance of the fra(X) 'gene' in apparently normal males who can subsequently transmit the gene to affected descendants (Sherman et al, 1984a).

In contrast to the above findings, Fryns (1984) reported a ratio of 1.82:1 for affected fra(X) males to normal males. The reason for this discrepancy is not known, but it may be due to an ascertainment bias. The ratio of Fryns (1984) was derived from an analysis of the progeny of carrier females. However, it was not stated whether carrier daughters of transmitting normal males were excluded from this analysis. All the daughters of fra(X) fathers are obligate fra(X) carriers whereas only 50% of daughters of

fra(X) mothers are carriers. The inclusion of progeny from female carriers of fra(X) fathers would result in an ascertainment bias in the ratio of affected males to normal males.

The deficit of affected males noted in the pedigrees of fra(X) families appears to be specific to this syndrome as no such deficit was observed in the pedigrees suggestive of fra(X) negative non-specific XLMR in the present investigation. However, the family history information obtained for fra(X) positive males was more extensive than that obtained for fra(X) negative males with non-specific XLMR. The apparent absence of a deficit of affected fra(X) negative males with non-specific XLMR needs to be examined further when additional family history information on these males becomes available.

The possible transmission of the fra(X) chromosome from apparently unaffected males has been previously reported (Webb et al, 1981; Brookwell et al, 1982; Rhoads et al, 1982; Fishburn et al, 1983; Van Roy et al, 1983; Kahkonen et al, 1983). The chromosomes of only two transmitting normal males have been examined to date. Webb et al (1981a) found the fra(X) in 26% of cells from an apparently normal male. Rhoads et al (1982) reported a case of an apparently normal transmitting male in whom the fra(X) was not detected cytogenetically. This male, however, was the son of an obligate carrier and the maternal grandfather of a retarded fra(X) male. The basis for the suggestion of fra(X) transmission

from apparently normal males stems from similar findings that retarded fra(X) males are related through fathers or maternal grandfathers to fra(X) families. In the present study, there are five cases which are suggestive of fra(X) transmission through an apparently normal male (Figures 19, 21, 25, 26 and 27).

Case 1

The pedigree of family Mn (Figure 19) shows an apparently normal male in generation IV (IV-2). However, this fra(X) positive male was less than one year of age at the time of testing. His mother (III-18) reported no milestone delay for development of the child. However, this would not exclude the manifestation of MR at a later date. Permission to perform testicular measurements was not given in this case.

Case 2

Another example of fra(X) transmission through an apparently normal male can be demonstrated in the pedigree of family W-B (Figure 21). The mentally retarded mother (I-8) of probands R.W. (II-10) and D.B. (II-16) had an apparently mentally normal brother (I-2). This man (I-2) had a normal daughter (II-3), who had a mentally retarded son, S.C. (III-4). Repeated fra(X) screening on S.C. did not demonstrate the fra(X). The medical records of S.C. showed MR of unspecified etiology.

Case 3

The pedigree of family C (Figure 25) also showed possible transmission of the fra(X) through a normal male (II-3). II-3 is the grandfather of probands IV-7 and IV-4. As proband IV-7 was born in 1917 and his eldest sister (IV-2) in 1907, it could be assumed that the majority of members of generation III were deceased and that none of generation II were alive. It was thus impossible to establish with certainty the existence of the fra(X) in individual II-3. If the grandfather (II-3) did not have the fra(X), then the presence of his retarded sibs would have been co-incidental and the marker would have been transmitted from the grandmother (II-2). If the grandfather (II-3) did have the fra(X), it remained unclear whether or not he was mentally normal. As he was a farmer in approximately the 1850's, a mild intellectual handicap would not necessarily have been observed in comparison with his more severely retarded siblings. If the proband's grandfather (II-3) did possess the fra(X), then all his sons would have been fra(X) negative and all his daughters would have been fra(X) positive. The probands' mother (III-6) was an obligate carrier. An attempt was made to trace the mother's sibs (III-1,2,3,-4,5) and their offspring. The probands' maternal uncles (III-3,4,5) appeared to be mentally normal. Thus it was possible that in this family, the fra(X) was transmitted in generation II through a "normal" male (II-3).

Case 4

The pedigree of family Bc (Figure 26) showed that transmission of the fra(X) occured possibly through the proband's (III-28) apparently normal grandfather (I-7). The grandfather's sister (I-2) had two mentally retarded sons (II-2,3) and an apparently normal daughter (II-1). This was suggestive of the fra(X) being transmitted through the proband's maternal grandfather. However, this could not be confirmed because contact with the proband's retarded second cousins (II-2,3) was lost. Alternatively, it was possible that the proband's grandmother (I-8) was heterozygous for the fra(X). This was not likely because the probability of her having four proven carrier daughters (II-4,5,8,11) and three normal sons was low (0.5^7 or 1 in 128).

Case 5

a) The fra(X) chromosome in family Fg (Figure 27) appeared to have been transmitted through the great-grandfather (I-1) of the initial proband R.Fg (IV-142). Of 11 children in generation II, 8 were obligate, unaffected carriers (II-2, 9, 11, 13, 15, 17, 19, 21) with proven fra(X) progeny; 1 daughter was mentally retarded (II-3); and 2 sons were normal (II-4, 6). The MR in II-3 was possibly a result of an 'active' fra(X). It could thus be assumed that all the daughters of I-1 were fra(X) positive and the sons fra(X) negative. This pattern of transmission was consistent with X-linked inheritance from a fra(X) male and

was to be expected if I-1 was a transmitting male. On the other hand, if R.Fg's (IV-142) great-grandmother (I-2) was heterozygous for the fra(X), the probability of her having 9 carrier daughters and 2 normal sons would have been 0.5^{11} (1 in 1,024) which was unlikely.

b) Another example of possible transmission through a normal male appeared again in the pedigree of family Fg (Figure 27). R.Fg.'s (IV-142) second cousin (IV-68) was a mentally retarded female. Her father (III-48) was apparently mentally normal. He also had a mentally normal daughter (IV-69). These individuals were not tested for the fra(X) since their whereabouts were unknown.

Fra(X) transmission does not occur according to the classical X-linkage. Not only does fra(X) transmission appear to occur through apparently normal males, it has also been suggested that the mothers of normal transmitting males appear to 'silence' the effects of the fra(X) by an unknown mechanism (Sherman et al, 1984b; Howard-Peebles and Friedman, 1984). Sherman et al (1984b) reported that the penetrance of mental impairment was higher in the offspring of normal daughters of transmitting males than in the offspring of normal mothers of transmitting males (Sherman et al, 1984a). This 'silencing' effect might be applicable to fra(X) family Bc (Figure 26) if I-7 was indeed a normal carrier. His carrier daughters (II-4,5,8,11) were all

unaffected as were his sibs (I-2,3,4,5,6). There was no information about the mother of I-7.

In contrast, a maternal 'silencing' effect was not applicable to fra(X) family C (Figure 25) since the mother (I-4) of the normal male carrier (II-3) was mentally retarded, as were several of his siblings (II-4, 6, 8, 10). His three daughters (III-1, 2, 6) and three sons (III-3, 4, 5) were mentally normal. Although all three daughters were postulated to be obligate carriers, only III-6 had fra(X) offspring.

The remaining cases of apparent male transmission of the fra(X) (Figure 19, IV-2; Figure 21, I-1; Figure 27, I-1, III-48) were uninformative regarding maternal 'silencing' effects. The male (IV-2) from family Mn (Figure 19) was an only child to date. The male from family W-B (Figure 21) was initially suspected to be a transmitting male because of the presence of a retarded grandson (III-4). However, this grandson (III-4) was fra(X) negative. No information was available regarding the mother of normal male I-1 in Family Fg (Figure 27). The daughters of male III-48 from family Fg (Figure 27) had not reproduced.

The presence of the fra(X) has not been established cytogenetically in any of the normal transmitting males in this study. It has only been postulated through analysis of the pedigrees. If it can be assumed that I-7 (Figure 26) and II-3 (Figure 25) are normal transmitting males, the

findings from the present study support the suggestion of Sherman et al (1984b) that normal mothers of transmitting males somehow 'silence' the fra(X) gene.

ii) Heterozygote Expression

Pedigree analysis of fra(X) families (excluding transmission from normal males) revealed 58 female members. If it is assumed that half of these females are heterozygotes, then 10 of a possible 29 (34.5%) carriers from the present study are expressing heterozygotes. All of the retarded females tested expressed the fra(X). Previous estimates of expressing heterozygotes ranged from 30-56% (Turner et al, 1980b; Brookwell et al, 1982; Fishburn et al, 1983; Sherman et al, 1984a). A review of the literature identified 217 cytogenetic fra(X) heterozygotes.* Of these, 80 (36.9%) were mentally deficient. This finding is in agreement with the present study.

Of the postulated 47.5 fra(X) negative female carriers with non-specific XLMR, 18 (37.9%) were expressing heterozygotes. It thus appears that a similar high rate of heterozygote expression is present in non-specific XLMR, both fra(X) positive and negative.

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*See references 1, 2, 3, 4-7, 8, 9, 12, 13, 15, 16, 17, 18, 20, 23, 24-27, 28, 29, 30, 31, 32, 35, 36, 37, 38-42; pp. 134-135.

Attempts to minimize ascertainment bias in this study were made by a determined effort to screen cytogenetically as many relatives of the proband as possible, especially younger female siblings. At least 2 cultures were obtained from all females who were initially determined to be fra(X) negative. Each culture was examined at least 2 and often 3 times by separate observers (200-300 cells) to confirm the absence of the fra(X) in these females.

A total of 56 females were screened cytogenetically for the fra(X). Twenty carriers were identified and an additional 6 with normal karyotypes were postulated to be obligate carriers. The finding of 26 carriers out of 56 females is in agreement with the theoretical approximation in which 50% (28/56) of females screened would be expected to be heterozygotes. Based on the finding that approximately 30% of fra(X) carriers manifest some degree of mental impairment, 9 of the 26 (30%) heterozygotes detected in the present survey would be expected to be mentally handicapped. Six MR heterozygotes were identified. A significant age effect was found among the mentally normal carriers (Figure 30b). Since the index cases in this study were all retarded males, an ascertainment bias might still be present due to the greater number of reproducing normal carriers as compared to affected carriers (Sherman et al, 1984a). The controversy concerning frequency of fra(X) expression and age in normal carriers might be resolved by:

1) a systematic fra(X) screening in a large newborn population and/or, 2) performing fra(X) screening on a large population of institutionalized females.

In the present study, the mean frequency of cells with the fra(X) in the carrier female group (10.6%) (Table 16) was significantly lower than that found in affected males (25.0%) (Table 15). Even if the six obligate carriers whose fra(X) frequency is 0% are excluded, the mean frequency of fra(X) in heterozygotes is 13.9%. While the reason for this difference is unknown, the lower percentage of fra(X) expression in heterozygotes as compared to hemizygotes may be the result of metabolic cooperation between cells with an active normal X chromosome and cells with an active fra(X) (Steinbach et al, 1983b). This cooperation would result in a suppression of fra(X) expression in heterozygotes.

Chudley et al (1983) reported a decline in fra(X) expression with increasing age in affected males. No such correlation was observed in the present study (Figure 30a). The present findings are in agreement with results of Brondum-Nielsen et al (1982).

No correlation was found in the present study between the percentage of fra(X) and intelligence either in hemizygotes (Table 15) or heterozygotes (Table 16). It can be seen that the more intelligent females and males do not have the lowest expression of the fra(X) and the reverse is also not true (Tables 15, 16; Chudley et al, 1983; Knoll et

al, 1984). This study showed that the morphology of examined chromosomes and quality of the culture greatly influenced the recorded percentage of expression. Fra(X) chromosomes were easier to detect on elongated chromosomes. It was also felt that since the fra(X) frequency could be influenced by external factors such as the quality of the culture and other variables that could affect culture conditions, the percentage of fra(X) expression should not be used as a prediction of intellectual level.

e) De Novo Mutations in the Fra(X) Syndrome

The 21 fra(X) probands identified in this survey represented 16 unrelated families since some brothers, half-brothers, and cousins resided in the same institution. They were randomly selected for cytogenetic analysis before pedigree information was available.

Three of the 16 families did not have any previous history of MR (Figure 12-14). The presence of the fra(X) in the proband in these families was postulated to be the result of de novo mutation. Information about one of these families (family K, Figure 14) was minimal as the proband's (II-3) immediate family died shortly after arriving in Canada from Czechoslovakia. No next of kin were listed. In another family (family R, Figure 12), the mother (II-4) of the proband (III-5) was examined for the fra(X) and was found to be negative. This, however, was inconclusive since several obligate carriers tested for the fra(X) were also

found to have a normal karyotype (Figure 30: I-8; Figure 31: III-7, 9). The third family (family L, Figure 13) was not available for fra(X) screening.

As mentioned earlier, a maximum estimate of fra(X) families from the present study which could have occurred as possible de novo mutations was 18.8% (3/16). Fryns (1984) found 37 families from an institutionalized survey of phenotypically selected males. In 21.6% (8/37) of these families, the proband was the only affected individual. From a computerized analysis of 100 fra(X) pedigrees, Sherman et al (1984a) determined that there were no sporadic fra(X) males. The values reported in the present study as well as those of Fryns (1984) and Sherman et al (1984a) are lower than the 33% (1/3) for sporadic cases predicted by Haldane (1935) for X-linked disorders. However, because the fra(X) syndrome appears not to follow classical X-linkage patterns, Haldane's (1935) estimation might not be applicable to the fra(X) syndrome (see section C3e in Literature Review).

It has been postulated that one-half (50%) of the mothers of probands with X-linked disorders (Francke et al, 1976), including the fra(X) (Herbst and Miller, 1980; Sherman et al, 1984a), are new mutants and that no affected males would be observed among her brothers. Of the 13 fra(X) families found in this survey with a positive history of MR (Figures 12-27), 4 (31%) had no maternal history of MR

(Figures 15-18). This frequency is lower than the expected value of 50%. If the estimation of Sherman et al (1984a) that there are no sporadic fra(X) males is correct, the three apparent 'sporadic' probands from the present survey (Figure 12-14) might be 'isolated' causes of fra(X) inherited from their obligate carrier mothers. If these three obligate carriers are included, the frequency of new mutation among the probands' mothers is 44% (7/16). This value is closer to the expected value of 50%.

It should be emphasized that an accurate family history is important to differentiate between possible de novo and inherited fra(X) mutations. An example can be illustrated in the pedigree of proband R.Fg. (IV-142, Figure 27). He was initially recorded as being abandoned with a distant aunt listed as the only next-of-kin. Follow-up studies on this aunt resulted in the pedigree shown in Figure 27 (personal communication, Public Health Clinic, Sudbury, Ontario). Included in this pedigree were the probands V-17 and V-18 (Figure 27). They were located in a separate institution and were at first thought to be a separate family. However, these probands (V-17,18) were related to fra(X) family Fg as a result of a consanguineous union between IV-94 and IV-95. Thus, institutional records can be inadequate in many cases, especially in the investigation of non-specific XLMR.

The controversy concerning the incidence of de novo fra(X) males can only be resolved through a large scale newborn survey in order to eliminate ascertainment bias regarding family history of MR. The presence of a differential sex mutation rate for the fra(X) males (see section C3e in Literature Review) could be determined through the use of RFLPs. The polymorphisms associated with the fra(X) chromosome could be traced through the mother to either the grandmother or grandfather of the proband. The establishment of segregation patterns of the X chromosome with an apparently de novo fra(X) mutation could be used to determine the origin of the fra(X) mutation, i.e., whether the mutation occurred in a male or female.

f) Limitations on Fra(X) Screening.

Two problems are encountered when screening metaphases for the presence of the fra(X) by solid-staining.

The first of these is the occurrence of telomeric breaks on C-group chromosomes (ie. #6, 8, 9) which with solid staining, resemble the X chromosome (Figure 5). To alleviate this problem, sequential Q-banding was used to verify the presence of a fra(X) (Figure 5). The frequency of such autosomal lesions was usually only 2-4%. Autosomal lesions are thought to occur via the same mechanisms which result in fragile sites, including the fra(X) (see section C3d in Literature Review). The mechanisms which result in the production of fragile sites, however, are thought to be

different from those causing chromosomal gaps and breaks (Vekemans et al, 1983).

The second difficulty of fra(X) diagnosis is the occurrence of Xq telomeric changes which are identical to the fra(X) in normal individuals (Popovich et al, 1982; Steinbach et al, 1982; Jenkins et al, 1983a). Some investigators have suggested that a minimal fra(X) incidence of 1-4% be used as a positive diagnosis of fra(X) (Jacobs et al, 1980; Herbst et al, 1981; Steinbach et al, 1982). Sutherland (1983), however, has suggested that any individual in whom even one fra(X) is seen to be regarded as having the fra(X) syndrome until this can be disproved by repeated chromosome studies or improved technology.

The results of fra(X) screening in two patients from the present study support the suggestions of Sutherland (1983). Two of the fra(X) positive males from the present study (A_1.D. and A_2.D., Table 14) had an initial fra(X) frequency of only 2% (Table 17). However, these cultures were characterized by an abundance of over-condensed spreads. In subsequent cultures with improved chromosome morphology, this frequency increased to 10 and 22% respectively (Table 17).

In contrast, the first culture of a fra(X) negative resident initially exhibited many gaps, breaks, and autosomal lesions. The fra(X) was also detected at an apparent frequency of 8%. The autosomal lesions included

telomeric breaks on the long arms of chromosome 6, 7, 8, and 9 in eight of the twenty cells examined. Three subsequent cultures from this individual showed no autosomal lesions and no fragile X site. The initial culture from this individual may represent: 1) an artifact of the culturing technique in the initial culture; 2) an over-population of cells resulting in exhaustion of nutrients causing a large number of gaps and breaks; or 3) a possible infection in the patient at the time the blood sample was taken.

Thus, the present findings lend strong support to Sutherland's (1983) suggestion that any instances of fra(X) be interpreted as positive, unless subsequent cultures do not substantiate the initial findings.

2 Sex Chromosome Anomalies (Figures 32-35)

The incidence of sex chromosome anomalies among [non fra(X)] MR (non-Down syndrome) males, both institutionalized and residing at home, has been found to be 0.8% (Rasmussen et al, 1982). The overall incidence (P_o) of sex chromosome anomalies in institutionalized MR (non-Down syndrome) males from the present survey was 0.4% (Table 20). A lower frequency of sex chromosome anomalies among MR males in institutions as compared to MR males residing in institutions and at home (Rasmussen et al, 1982), has also been reported previously (Table 2). This lower incidence of sex chromosome anomalies in the present and previous surveys can be explained by different admission policies in various

regional institutions. A higher incidence of sex chromosome anomalies is found in mildly or moderately retarded populations than in the more severely affected population (Court-Brown, 1969; Jacobs et al, 1978). The institutionalized population examined in the present and previous surveys may consist of fewer mildly or moderately retarded males as these individuals are more likely candidates for group homes than for institutions.

Of the 2 individuals with sex chromosome anomalies detected in this survey (Table 18), one was a 47,XXY° and the second was a 47,XYY. The finding of micro-orchidism in the Klinefelter patient is in agreement with previous reports (Paulsen, 1974). The 47,XYY individual was macro-orchid and tall (184 cm) (see Appendix 1). It has been mentioned earlier (see section B1 in Discussion) that there is no correlation between macro-orchidism and height.

3. Autosomal Chromosome Anomalies

a) Cri-du-chat syndrome (Figures 36-41)

The overall frequency (P_o) of the cri-du-chat syndrome found in this survey was 0.9% (Table 20). Previously reported frequencies for the 5p- syndrome in institutions ranged from 0.18-0.59% (Niebuhr et al, 1978; Ally and Grace, 1979; Nielsen et al, 1982). Higher frequencies of the 5p- syndrome have been found in retarded individuals with an IQ below 50 (Niebuhr, 1978). The high frequency found in this study as compared to values reported

previously could be the result of:

1) The sample population studied. The present study included only non-Down syndrome males as compared to Down and non-Down syndrome patients of both sexes in other studies. The exclusion of Down syndrome patients in this study may have resulted in a high concentration of individuals with other chromosome anomalies. The exclusion of females in the present study is not likely to result in the high frequency reported because an excess of females with the 5p- syndrome has been found (Niebuhr, 1978).

2) Random variation.

3) Regional differences in institutional policy. Placement of mildly or moderately retarded individuals into group homes would result in a high concentration of severely retarded in institutions. This would result not only in a decrease of reported sex chromosome anomalies (see section above) (Court-Brown, 1969; Jacobs et al, 1978), but also a concomittant increase in disprders more prevalent among the severely retarded, such as the 5p- syndrome (Niebuhr, 1978).

b) Balanced Translocations (Figures 42-50)

A total of 6 (P_o= 1.1%) (Table 20) apparently balanced translocations were found in the present survey. The clinical features of these patients are described in Appendix 1. The mean frequency of balanced rearrangements among institutionalized individuals, if Down syndrome patients were excluded, is 0.7% (Table 2). The high

frequency of translocations found in this study is probably the result of random variation. Rasmussen et al (1982) and Jacobs et al (1978) also reported frequencies of 1.1% and 1.3% respectively if Down syndrome males were excluded. The incidence of balanced translocations found in the present study and in the studies of Jacobs et al (1978) and Rasmussen et al (1982) is approximately 6 times greater than the 0.2% reported for newborn surveys (Nielsen and Sillesen, 1975).

In general, balanced chromosomal rearrangements do not lead to phenotypic abnormalities unless there are qualitative or quantitative alteration of the genetic material. The most frequent type of chromosome rearrangement in human populations is Robertsonian translocation or centric fusion (Vogel and Motulsky, 1979) and the most common of these are t(13q14q) and t(14q21q) (Jacobs, 1974). Jacobs (1974) observed an increase in de novo rather than familial arrangements among the retarded. Funderbunk et al (1977) reported that this increase involved primarily nonRobertsonian translocations.

Of the translocations found in this survey, three were familial [45,XY,-13,-14,+t(13q14q); 46,XY,t(14;15)(q32;q13) (Figures 44,45)]; two were de novo [46,XY, t(5;14)(q22;q31) (Figures 47,48); 46,XY,t(16;22)(p12;q13) (Figures 49,50)] and no information was available on the last [46,XY, t(7;16)(q11;p13)(Figure 51)].

Of the three familial cases of apparently balanced translocations, two [46,XY,t(14;15)(q32;q13) (Figure 44)] were found to be identical and were segregating in a pair of brothers (Figure 45). The father of these two probands was also mentally retarded and was found to have the same apparently balanced translocation. In addition, all three family members who carried the translocation showed abnormal phenotypic features such as large ears, prominent sclera visible below the iris, and notched teeth. A cleft palate was present in both probands as well as in some maternal relatives. As the karyotypes of both parental grandparents were normal, the translocation likely arose as a de novo event during gametogenesis in one of the grandparents.

A testicular biopsy specimen from the father of these two probands showed apparent complementary pairing along all four chromatids (Figure 47) (Dr. F.R. Sergovich, personal communication). The translocation thus appears to be balanced, at least at the present level of resolution. The clinical phenotype associated with this translocation may be due to a position effect involving the translocated segments.

Only one Robertsonian translocation was detected: 45,XY,-13,-14,+t(13q14q) (Figure 42). Its occurrence is compatible with random expectations. This finding is in agreement with Funderbunk et al (1977) who found that the majority of rearrangements among the retarded are

non-Robertsonian. This translocation was segregating through the maternal side. Both maternal grandparents were deceased so it was not possible to determine the exact origin of the rearrangement. The proband was the only retarded individual with this translocation. A paternal female first cousin was also mentally retarded. The mental status of the proband might thus be the result of paternal genetic influence with the translocation being coincidental.

c) 46,XY,der(8q+)?

This karyotype of proband T.W. showed an extra segment attached to the terminal portion of 8q (Figure 52). The origin of this partial trisomy could not be identified. The karyotypes of the proband's parents and sisters were normal (Figure 53). The clinical features of this patient are described in Appendix 1.

d) Partial trisomy 21

All institutionalized males known to have Down syndrome were excluded from this study. On the admission clinical assessment of MR patient S.Cn., no symptoms typical of Down syndrome were described (see Appendix 1). He was thus included in this survey by random selection of non-Down residents.

S.Cn. was found to carry the chromosome abnormality, 46,XY,dup(21)(q22) (Figures 54, 55). The karyotypes of both parents were normal for chromosome 21, although the mother was a 46,XX/47,XXX/48,XXXX mosaic. Thus the

dup(21)(q22) in the present proband appears to have arisen as a de novo event. De novo duplication within the same chromosome in abnormal individuals is hypothesized (Vogel et al, 1978) to result from either a translocation between the homologous chromosome during meiosis or an insertion or exchange of chromatids that occurred within an inversion loop during parental meiosis.

The development of chromosome banding techniques allowed the identification of partial trisomy 21 in patients with features of Down syndrome who had normal karyotypes with solid staining. Studies of these patients' karyotypes revealed that the additional chromosomal material responsible for the Down syndrome phenotype was in segment 21q22 (Pueschel et al, 1980; Turleau et al, 1980; Hagemeijer and Smith, 1977).

Superoxide dismutase (SOD) is an enzyme which has been found to show a gene dosage effect of increased activity by 150% in nucleated trisomy 21 cells (Feaster et al, 1977; Francke, 1981). [SOD catalyzes the dismutation of superoxide anions (0_2^-) to hydrogen peroxide and molecular oxygen (Minami and Yoshikawa, 1979) and is implicated in the prevention mechanism against methemoglobin formation by superoxide anions.] The approximate localization of the SOD gene has been suggested to be in or just proximal to band 21q22.1 (Poisonnier et al, 1976; Sinet et al, 1976; Yamamoto et al, 1979; Leschot et al, 1981;

Mattei et al, 1981; Habedank and Rodewald, 1982). However, a partial trisomy for 21q21 → q22.2 with normal SOD levels (Jenkins et al, 1983b) raises the question as to the precise location of the SOD gene within the 21q22 band. The normal SOD levels found in the present proband and the diminished clinical features suggest a partial duplication of band q22, possibly below 21q22.1.

e) Distal 10p deletion syndrome

In the present study, one proband (K.C.) (Figure 58) was found to have a deletion of chromosome segment 10p13 → 10pter (Figure 57). The parents of K.C. were reported to be of low intelligence and would not consent to chromosome analysis. The clinical features of proband K.C. are described in Appendix 1.

To date, only eleven children with a partial deletion of the short arm of chromosome 10 have been identified by chromosome banding techniques (Shokeir et al, 1975; Francke et al, 1975; Berger et al, 1977; Bourrouillou et al, 1981; Fryns et al, 1981; Juberg et al, 1981; Klep-dePater et al, 1981; Gencik et al, 1983; Elstner et al, 1984). The most common cause for partial monosomy 10p is through a de novo deletion of the segment 10p13 → pter (Francke et al, 1975; Shokeir et al, 1975; Berger et al, 1977; Klep-dePater et al, 1981; Gencik et al, 1983; Elstner et al, 1984). No specific loci have yet been assigned to this region (McKusik, 1983).

f) 47,XY,+inv dup(15)(p13-q12)

Three cases of dicentric inv dup(15) were found in the present survey (P_o= 0.8%) (Table 20).

All three patients had the 47,XY chromosome complement which was the result of the presence of a supernumary bi-satellited marker (Figures 59-61). There was no evidence of mosaicism. G-, C-, and NOR-banding revealed that the extra chromosome was dicentric and composed of two D or G group short arms which were separated by a small euchromatin-like band (Figures 62, 63). The MG/DAPI technique (Figure 64) produced prominent subterminal fluorescent bands on either end of the marker chromosome. On the basis of these findings, the extra chromosome was interpreted to be a dicentric inversion duplication of chromosome 15 (Table 18). Clinical features of the patients with the inv dup (15) from the present study are described in Appendix 1.

A dicentric inv dup (15) is postulated to occur from a U-type of exchange resulting from crossover mistakes during meiosis (Schreck et al, 1977; Wisniewski et al, 1979). Non-disjunction must accompany this mechanism to account for the presence of the additional 47th chromosome.

Connor and Gilmore (1984) have reported the presence of a maternal age effect for de novo cases of inv dup (15). This suggests that, as in trisomy 21, the mother is the usual source of the extra chromosome in inv dup (15). In the present study, 2 of the 3 probands with inv dup (15)

(R.McF., Figure 64; C.V., Figure 66) had mothers who were ≥ 35 years at the time of delivery (see Appendix 1). The parents of probands R.McF. and C.V. were not available for chromosome studies. The parents of only one proband (H.W.) (Figure 65) were available for chromosome analysis. The karyotypes of these parents were normal. NOR-staining and Q-banding did not reveal the presence of any chromosome polymorphisms. Thus, the origin of the inv dup (15) in H.W. could not be determined.

There do not appear to be any severe physical malformations in patients having inv dup (15) (Schreck et al, 1977; Wisniewski et al, 1979). Stetton et al (1981) reported one case of inv dup (15) which was detected prenatally. This additional chromosome was also present in the lymphocytes of the mother who had a normal clinical phenotype. Knight et al (1984) observed a mosaic inv dup (15) in a father and daughter who were also normal phenotypically. Chromosome polymorphisms suggested that the inv dup (15) arose by nondisjunction at the first meiotic division in the paternal grandmother.

E. Testicular Volume as a Diagnostic Criterion in MR

The most intriguing finding from the present investigation is the high incidence of macro-orchidism among institutionalized (non-Down syndrome) males (Pozsonyi et al, 1981). While the etiology of macro-orchidism among MR males remains generally unknown, the preferential association of

macro-orchidism with the fra(X) syndrome suggests that the mechanism(s) involved in the expression of the fra(X) may be associated with the pathogenesis of the macrotestes. The finding that the testes of fra(X) males tend to exceed volumes of 40 ml [as compared to the testes of fra(X) negative males with non-specific XLMR] supports this hypothesis. Further clinical and biochemical studies are indicated for MR males with macro-orchidism.

The presence of apparently normal males in a fra(X) family poses a dilemma for the genetic counsellor. Although the fra(X) chromosome is not always demonstrated cytogenetically in cells from asymptomatic fra(X) males, these males can nevertheless transmit the fra(X) 'gene' to their offspring (Rhoads et al, 1982). The usage of RFLPs will undoubtedly resolve this dilemma in the future. At present, however, the clinician needs a more rapid method of assessment.

It is also possible that macro-orchidism may be a useful clinical marker for apparently normal fra(X) males. The incidence of macro-orchidism among normal males from fra(X) families, however, has not been examined extensively. To date, the testicular volume of only one such normal, transmitting fra(X) male has been reported (Rhoads et al, 1982). This Japanese male had testicular volumes of 7.9 ml (right testis) and 13.0 ml (left testis). Both these values would be considered micro-orchid in the present study.

However, this reduced testicular volume may be the result of his ethnic background as there is some evidence that testicular volume differs among ethnic groups (Turner and Jacobs, 1984). Schonfeld (1943) found that the mean testicular volume of Japanese men was approximately 12 ml as compared to 17-19 ml for Caucasian males (Laron and Zilka, 1969; Zachmann et al, 1974). Affected fra(X) males from the same family had testicular volumes (right and left testes) ranging from 14.7-37.7 ml (Rhoads et al, 1982). Breen et al (1981) reported a mentally normal 12 1/2 year old fra(X) male with bilateral testicular enlargement (right testis, 38.2 ml; left testis, 31.9 ml). Testicular measurements were not available for any of the apparently normal fra(X) males from the present study. It is suggested that a more concerted effort should be made by investigators of the fra(X) disorder to determine the testicular volumes of all males from fra(X) families.

The results from the present study indicate that patients with testicular volumes of 15 ml or less are associated with a high incidence of spastic/paralytic disorders. Although micro-orchidism was shown to be of little clinical value in the assessment of MR males with possible chromosome abnormalities, reduced testicular volume can be used as a clinical marker for possible neurological damage. Detailed neurological assessment of MR males with testicular volume $\leq$ 15 ml [such as that conducted by Opitz

et al (1978)] may reveal possible causal factors associated with their MR.

It has been suggested from results in the present study that enlarged testicular volume may be a common feature of MR males without spastic/paralytic disorders. The subdivision of institutionalized males into two categories based on the presence or absence of spastic/paralytic disorders results in adult MR males whose mean testicular volumes are 12.8 $\pm$ 7.1 ml and 26.0 $\pm$ 20.6 ml respectively. Thus, the exclusion of institutionalized males with spastic/paralytic disorders results in a population of MR males whose mean testicular volume is greater than the mean of the normal male population (17-19 ml) (Laron and Zilka, 1969; Zachmann et al, 1974). Enlarged testicular volume may therefore be a common feature of MR males who do not have a history of neurological damage.

The apparent delay in testicular development observed in MR males from the present study needs to be further investigated. The rate of testicular enlargement should be determined for adolescent MR males with and without the presence of spastic/paralytic disorders. Concomittant studies should also be initiated on a control population of normal adolescent males, with and without such neurological disorders.

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VI SUMMARY

(1) The testicular volumes of 878 institutionalized (non-Down syndrome) males were determined by using sliding calipers and the formula, $V = \frac{\pi}{6} \times l \times w^2$ (Cantu et al, 1976). Macro-orchidism ($V \geq 25$ ml) was found in 20.8% of adult male residents, micro-orchidism ($V \leq 15$ ml) in 43.1%, and normal testicular volume ($15 < V < 25$ ml) in 33.3%. Bilateral cryptorchidism was found in 2.8% of patients. The mean adult testicular volume of MR males was found to be 19.5 ± 15.8 ml. The range of 0.6 to 177 ml. Testicular development among institutionalized males from the present study was delayed as compared to the normal male population.

(2a) The overall, adult mean height of institutionalized (non-Down syndrome) males was 164.1 ± 11.9 cm. Micro-orchid males were found to be significantly shorter (161.2 ± 13.1 cm) than males with either macro-orchidism (167.1 ± 11.7 cm) or normal testicular volume (165.2 ± 9.3 cm).

(b) A significant correlation was found between height and micro-orchidism. No correlation was found between height and testicular volumes ≥ 15 ml. These findings indicate that testicular volumes ≤ 15 ml are influenced to some extent by general body size but that macro-orchidism ($V \geq 25$ ml) is not associated with a concomittant increase in body size.

(3a) As determined from available medical records, the overall frequency (P_o) of spastic/paralytic disorders (cerebral palsy, spasticity, hemiplegia, paraplegia, and quadriplegia) among institutionalized (non-Down syndrome) males was found to be 23.7%. Micro-orchid males had a significantly higher incidence of spastic/paralytic disorders (39.8%) than males with either macro-orchidism (4.2%) or normal testicular volume (15.6%).

(b) It is suggested that the presence of such neurological damage may result in impaired testicular function with subsequent loss of volume.

(c) Micro-orchidism ($V \leq 15$ ml) can thus serve as a clinical indicator of possible neurological damage in MR males.

(4) The most frequently detected chromosome abnormality among the institutionalized (non-Down syndrome) males from the present study was the fra(X) syndrome ($P_o = 3.1\%$). A significantly higher proportion of macro-orchid males (10.3%) were found to have the fra(X) syndrome as compared to males with either micro-orchidism (1.2%) or normal testicular volume (1.2%). This preferential association of macro-orchidism with the fra(X) establishes the use of enlarged testicular volume (especially $V \geq 40$ ml) as a clinical marker in the diagnosis of the fra(X) syndrome.

(5a) There was no significant difference in the distribution of fra(X) negative non-specific XLMR among males with macro-orchidism (10.9%), micro-orchidism (9.7%), and normal testicular volume (9.1%).

(b) The frequency of heterozygote expression was similar in both fra(X) positive and negative non-specific XLMR.

(6) Ultrasound analysis of macro-orchid testes from both fra(X) positive and negative males revealed no consistent pathological abnormality, although in some cases a prominent ductal pattern was observed; others showed a small amount of fluid in the tunica vaginalis.

(7) The overall frequency (P_o) of non-fra(X) chromosome abnormalities was 3.6%. Although micro-orchid males showed a higher frequency of non-fra(X) chromosome abnormalities (4.2%) than either males with macroorchidism (2.4%) or normal testicular volume (3.0%), this difference was not statistically significant. Therefore, micro-orchidism is of little use as a clinical marker for the evaluation of MR males with possible chromosome abnormalities.

(8) The chromosome abnormalities detected in this survey included the cri-du-chat syndrome; the 47,XY,+inv dup(15)(p13→q12); apparently balanced translocations; sex chromosome anomalies (47,XXY and 47,XYY); a 46,XY, dup(21)(q22); a 46,XY,del(10)(p13→pter); and a 46,XY,der(8q+)?.

(9) Balanced translocations were found at an overall frequency (P_o) of 1.1%. There was no significant difference in the distribution of balanced translocations among the three testicular volume groups.

(10) The presence of a supernumary inv dup(15) chromosome was found to be almost as common a cytogenetic anomaly among institutionalized males as the cri-du-chat syndrome.

Figure 1. Diagrammatic representation of the fragile X site.

→, Xq28: Region of the 'fra(X)' site. (Paris Conference, 1971)

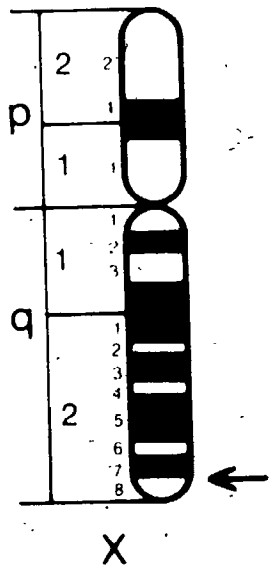

p
q
2
1
1
2
X

Figure 2. The area of folate metabolism involved in fragile site expression (Erbe, 1975; Scott and Weir, 1981).

The enzymes controlling the various reactions are (1) methionine synthetase, (2) glutamate formiminotransferase, (3) serine hydroxymethyltransferase, (4) methylene-THF reductase (5) formimino-THF cyclodeaminase, (6) dihydrofolate reductase, (7) thymidine kinase, (8) thymidylate synthetase. THF tetrahydrofolate; DHR, dihydrofolate; dUMP, deoxyuridine monophosphate; dTMP, deoxythymidine monophosphate.

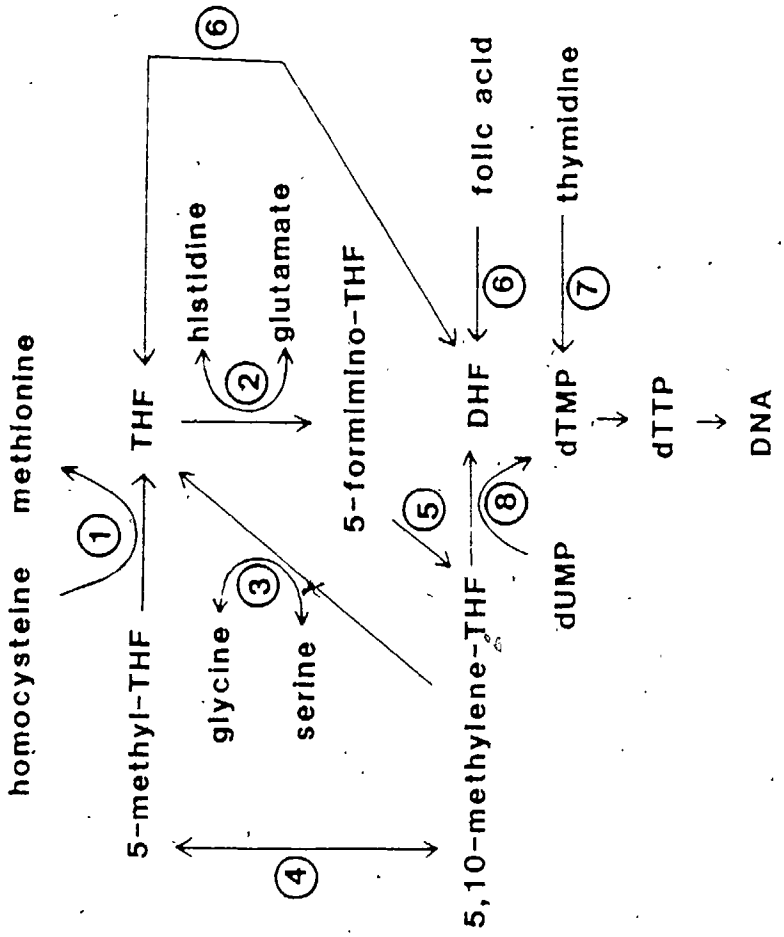

homocysteine
methionine
5-methyl-THF
THF
glycine
serine
histidine
glutamate
5-formimino-THF
5,10-methylene-THF
DHF
folic acid
thymidine
dUMP
dTMP
dTTP
DNA
1
2
3
4
5
6
7
8

Figure 3. The known fragile sites, other than Xq28.

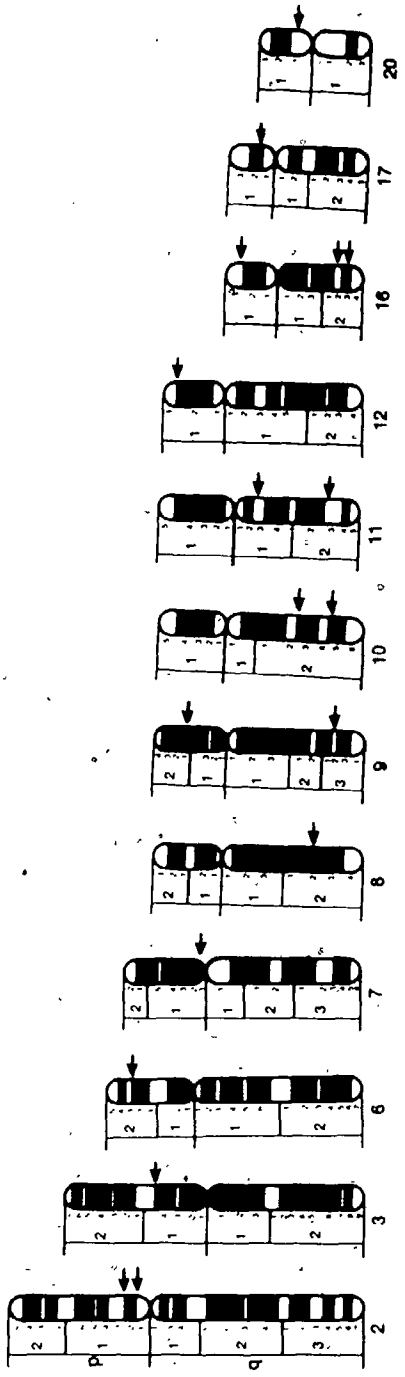
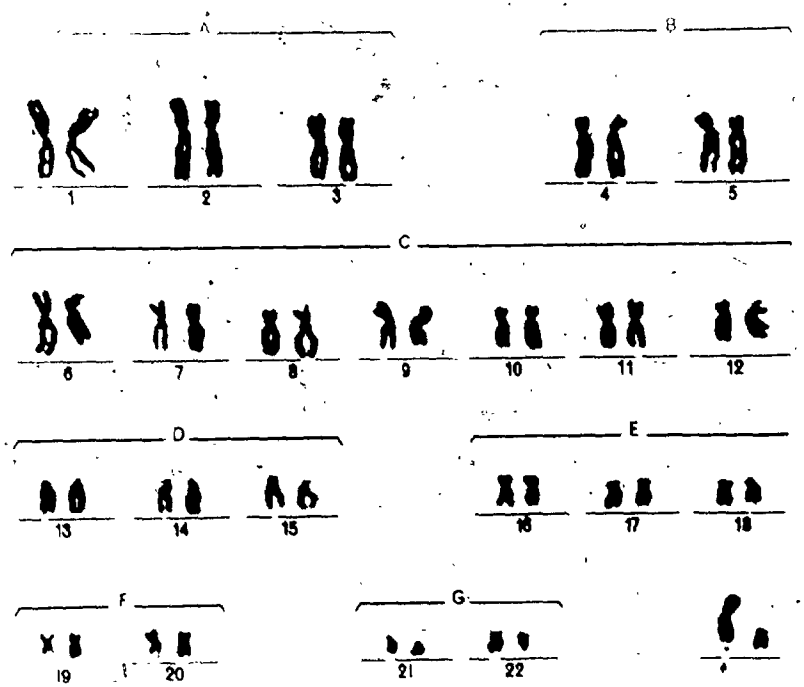
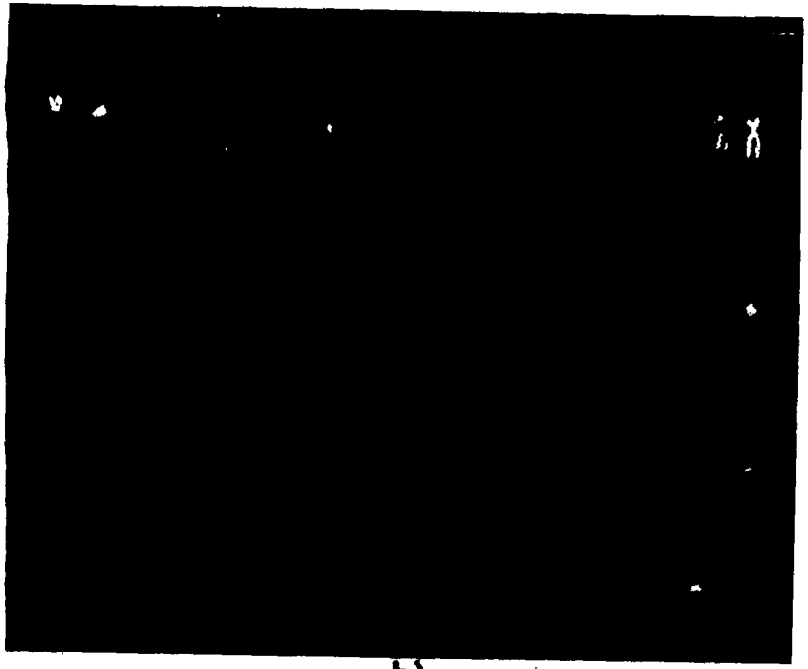

20
17
16
12
11
10
9
8
7
6
3
2
p
q

Figure 4. Microscopic detection of the fra(X).

a) with solid staining using Giemsa
b) with quinacrine fluorescence (Q-bands)



a)

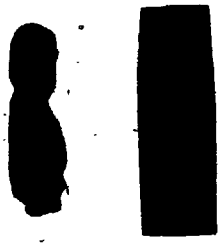


b)

Figure 5. Common Autosomal Lesions.

a) chromosome #6
b) chromosome #8
c) chromosome #9

a)



b)

c)

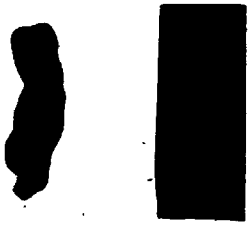


Figure 6. Testicular volume (ml) versus age (yr) in institutionalized males.

———, mean testicular volume
- - -, ± 1 standard deviation

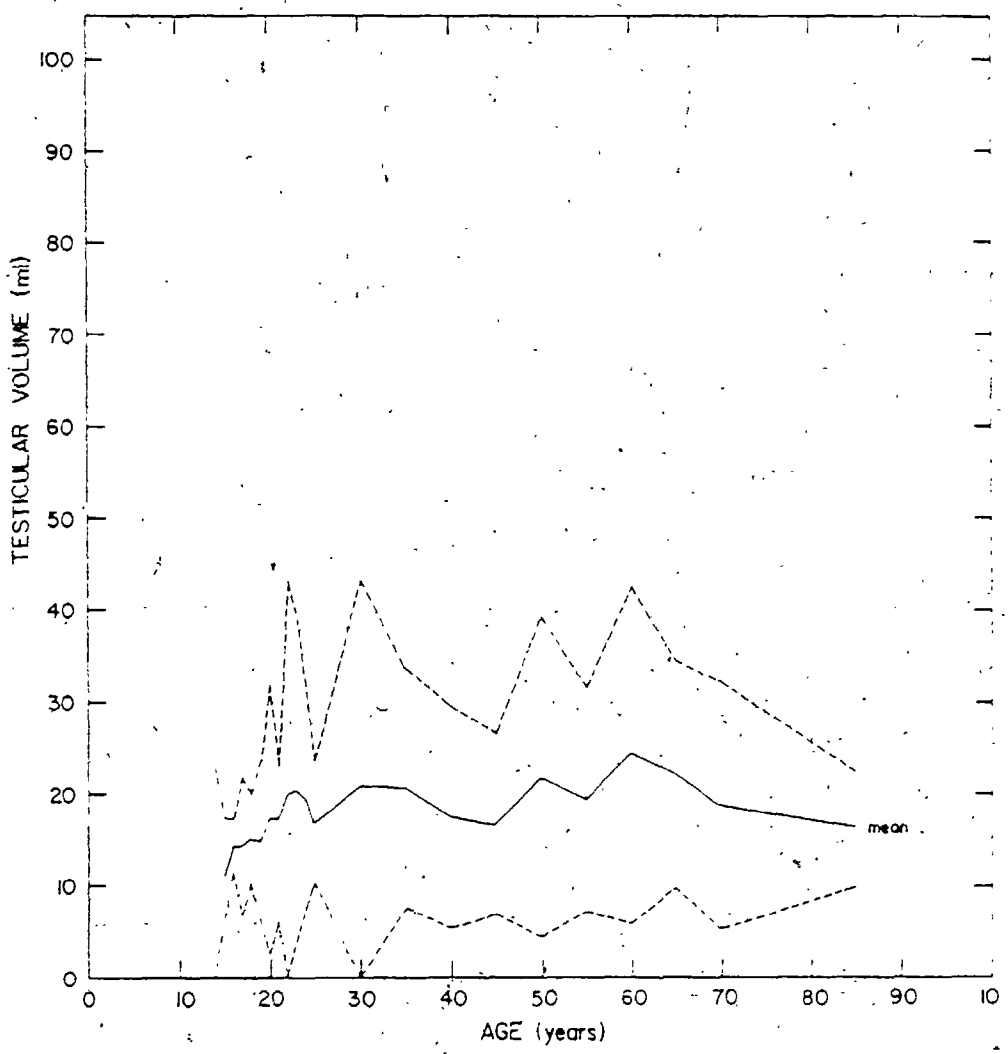

TESTICULAR VOLUME (ml)
100
90
80
70
60
50
40
30
20
10
0
0
10
20
30
40
50
60
70
80
90
10
AGE (years)
mean

Figure 7. Testicular development

 , Schonfeld (1943)

 , Hansen and With (1952)

 , Rundle and Sylvester (1962)*

 , Dooren et al (1963)

 , Prader (1966)

 , Zilka and Laron (1969)

 , Zachmann et al (1974)

 , 90th percentile Zachmann et al (1974)

 , present study*

* mentally retarded populations

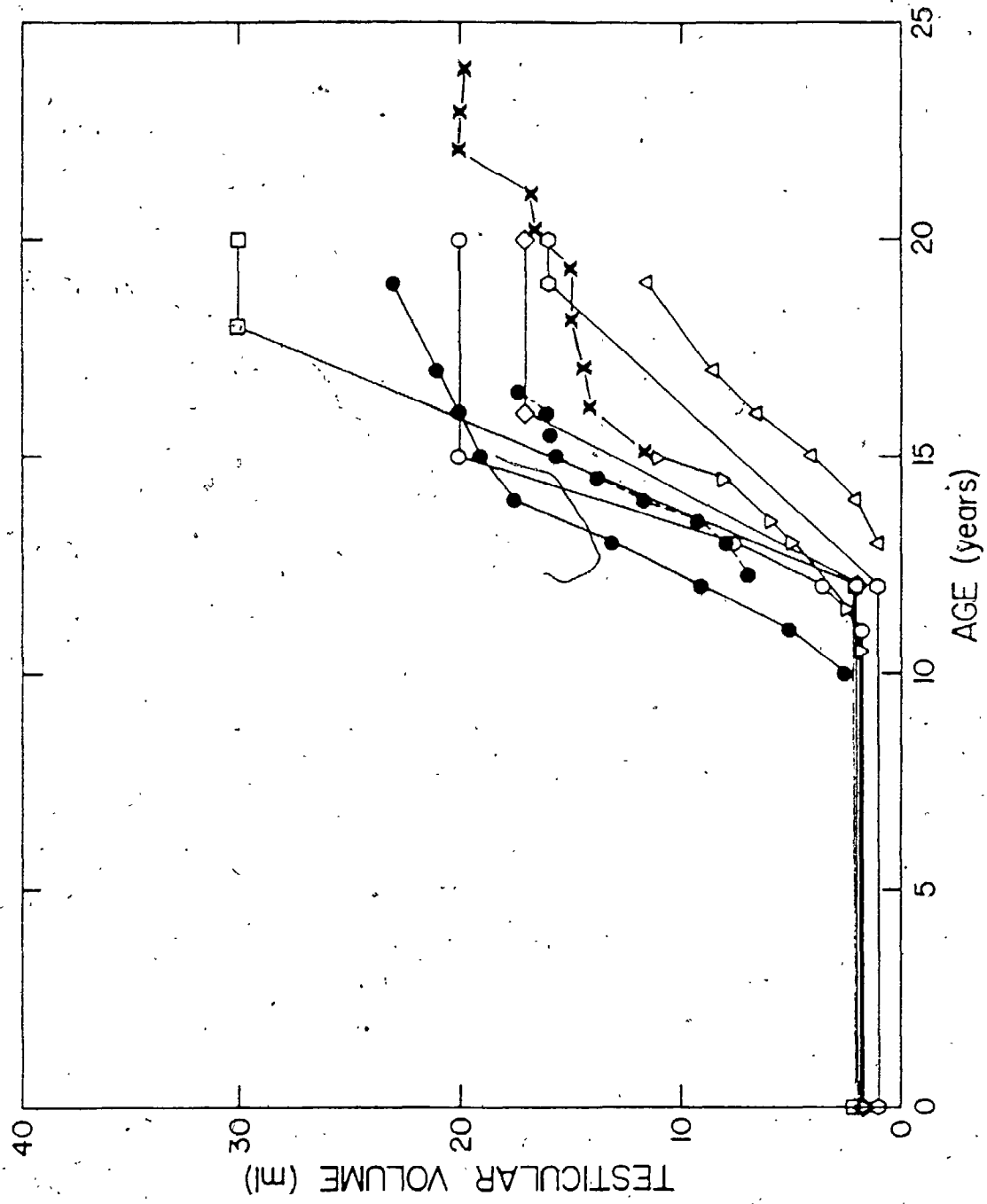

TESTICULAR VOLUME (ml)
40
30
20
10
0
0
5
10
15
20
25
AGE (years)

Figure 8. Histogram showing the distribution of testicular volume (ml) among institutionalized males.

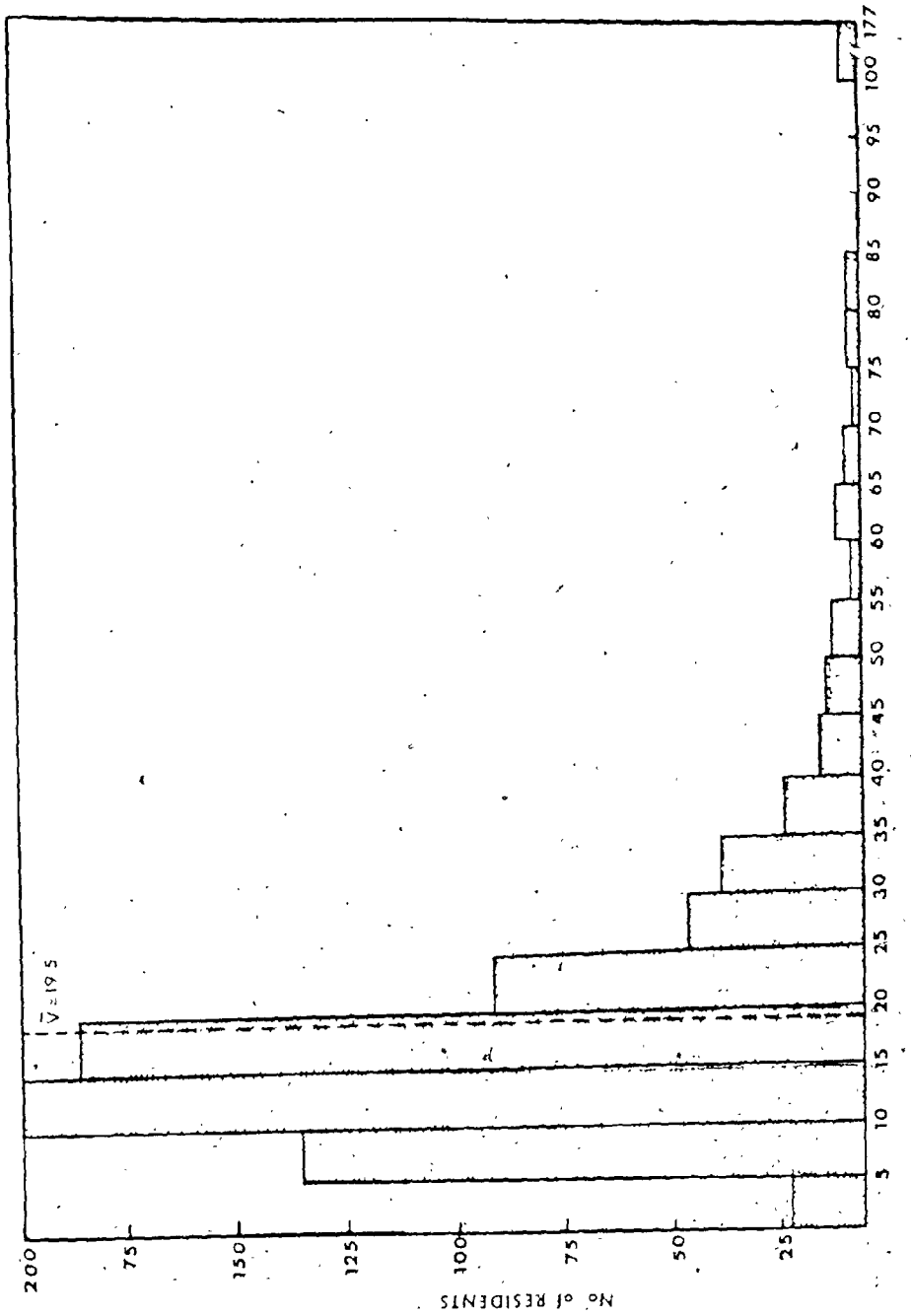

No. of RESIDENTS
200
75
150
125
100
75
50
25
TESTICULAR VOLUME (ml)
5
10
15
20
25
30
35
40
45
50
55
60
65
70
75
80
85
90
95
100
177
V=19.5

Figure 9. Non-specific X-linked MR [fra(X) negative] with macro-orchidism.

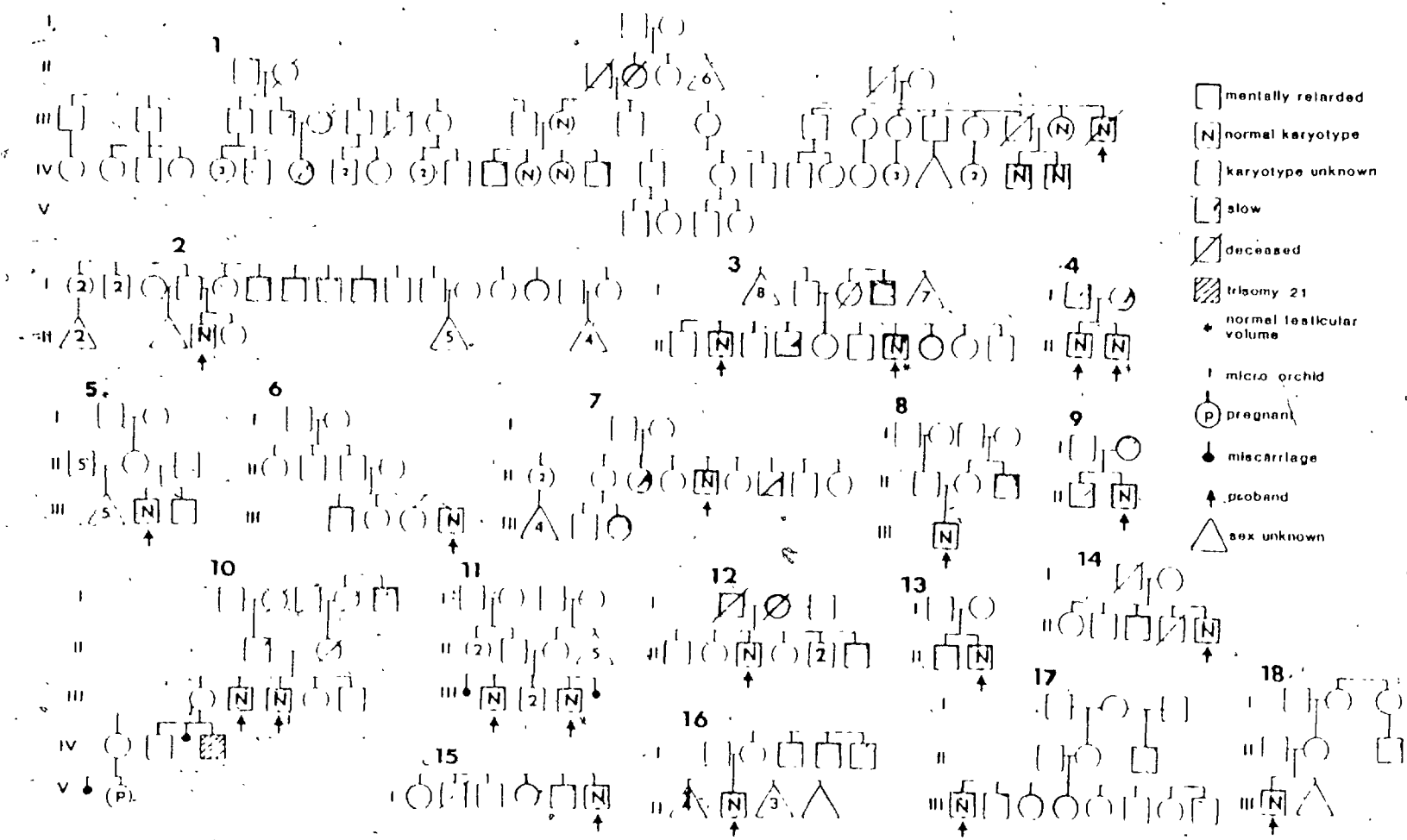

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
mentally retarded
normal karyotype
karyotype unknown
slow
deceased
trisomy 21
normal testicular volume
micro orchid
pregnant
miscarriage
proband
sex unknown

Figure 10. Non-specific X-linked MR [fra(X) negative] with micro-orchidism.

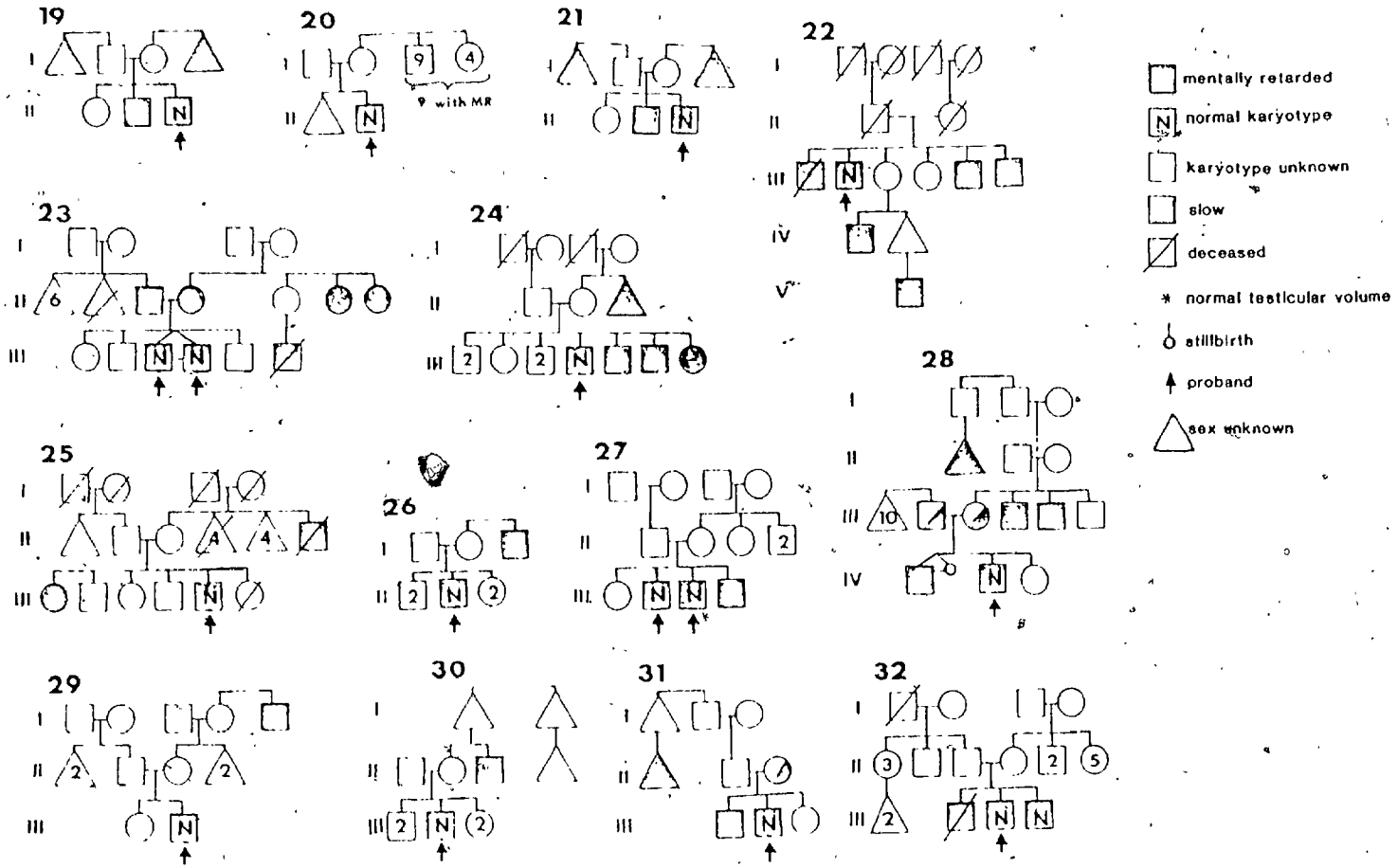

19
20
9 with MR
21
22
23
24
25
26
27
28
29
30
31
32
mentally retarded
normal karyotype
karyotype unknown
slow
deceased
normal testicular volume
stillbirth
proband
sex unknown

Figure 11. Non-specific X-linked MR [fra(X) negative] with normal testicular volume.

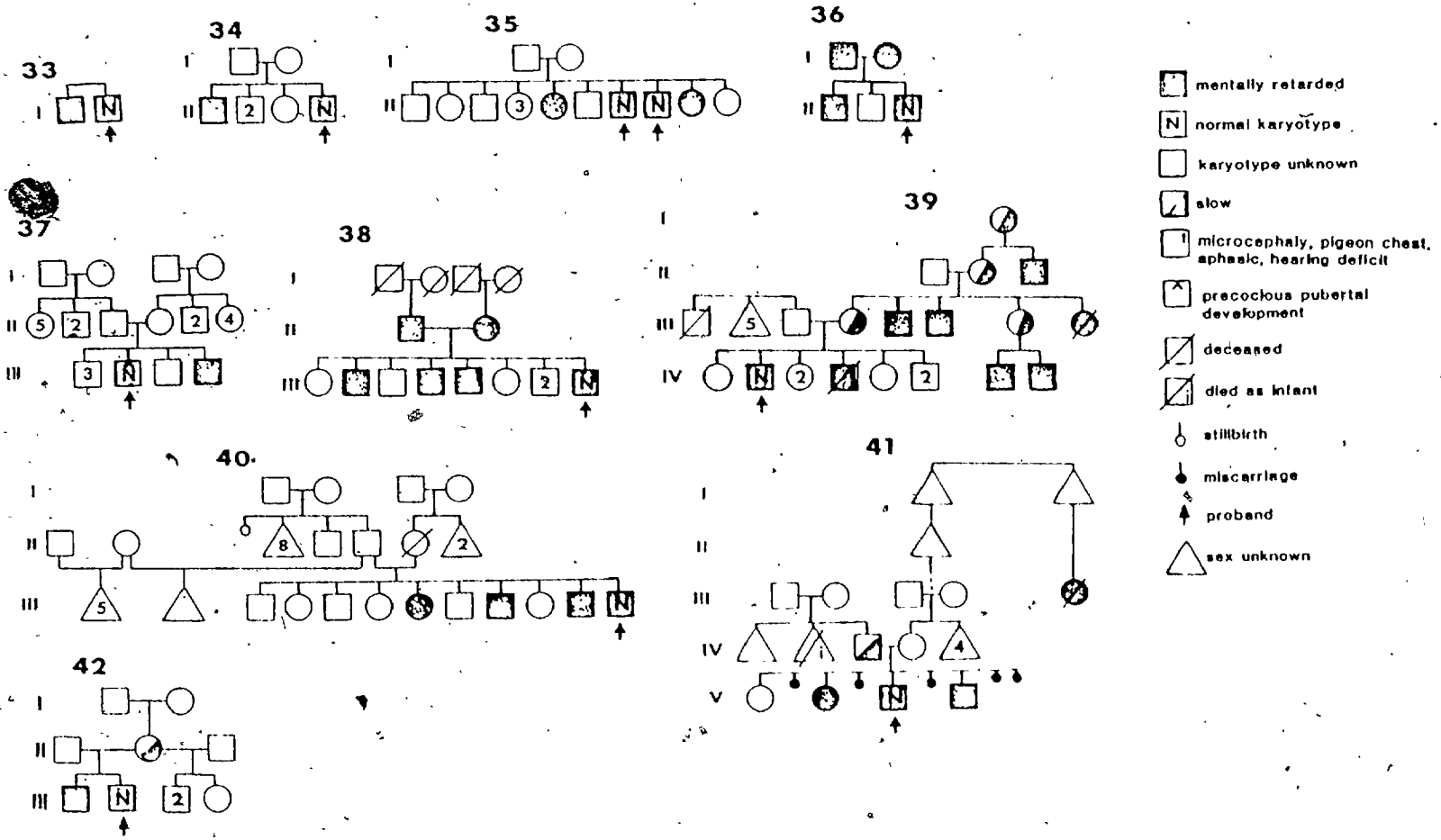
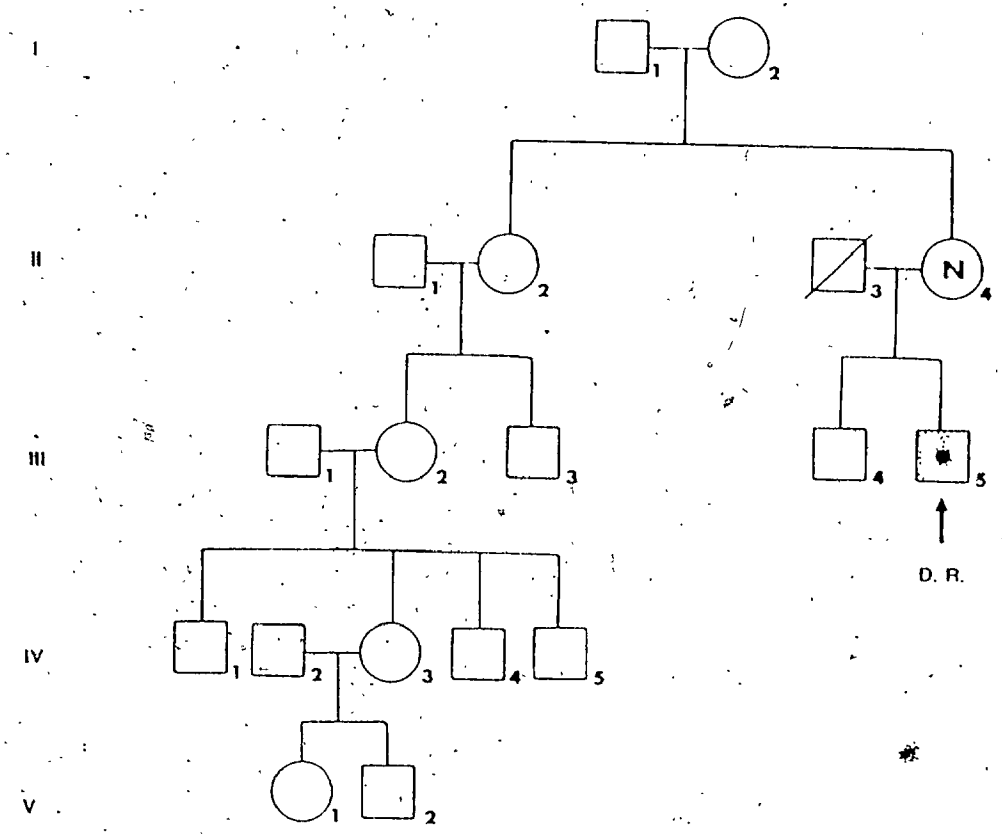

33
34
35
36
37
38
39
40
41
42
mentally retarded
normal karyotype
karyotype unknown
slow
microcephaly, pigeon chest, aphasic, hearing deficit
precocious pubertal development
deceased
died as infant
stillbirth
miscarriage
proband
sex unknown

Figure 12. Pedigree of fra(X) family 'R.

PEDIGREE OF FRA(X) FAMILY R.



mentally retarded
fra(X) positive
N normal karyotype
karyotype unknown
deceased
proband

Figure 13. Pedigree of fra(X) family L.

PEDIGREE OF FRA(X) FAMILY L.

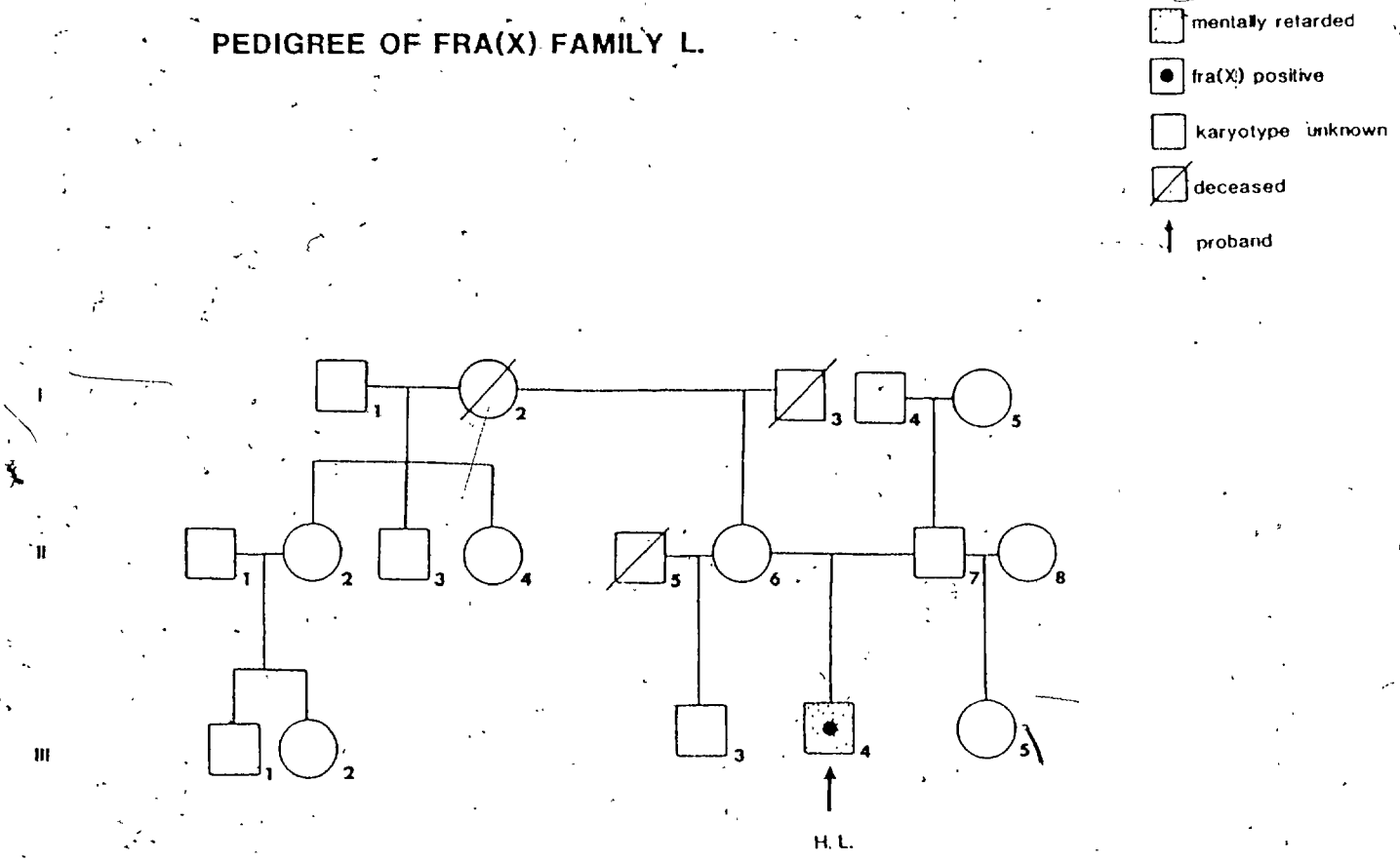
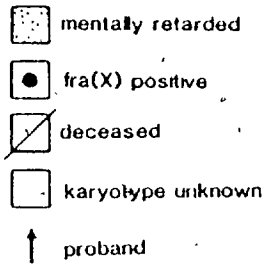


Figure 14. Pedigree of fra(X) family K.

PEDIGREE OF FRA(X) FAMILY K.



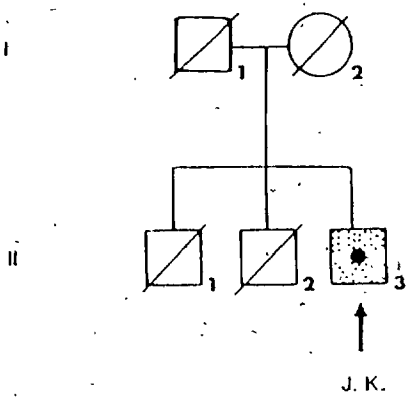


Figure 15. Pedigree of fra(X) family Wk.

PEDIGREE OF FRA(X) FAMILY Wk.

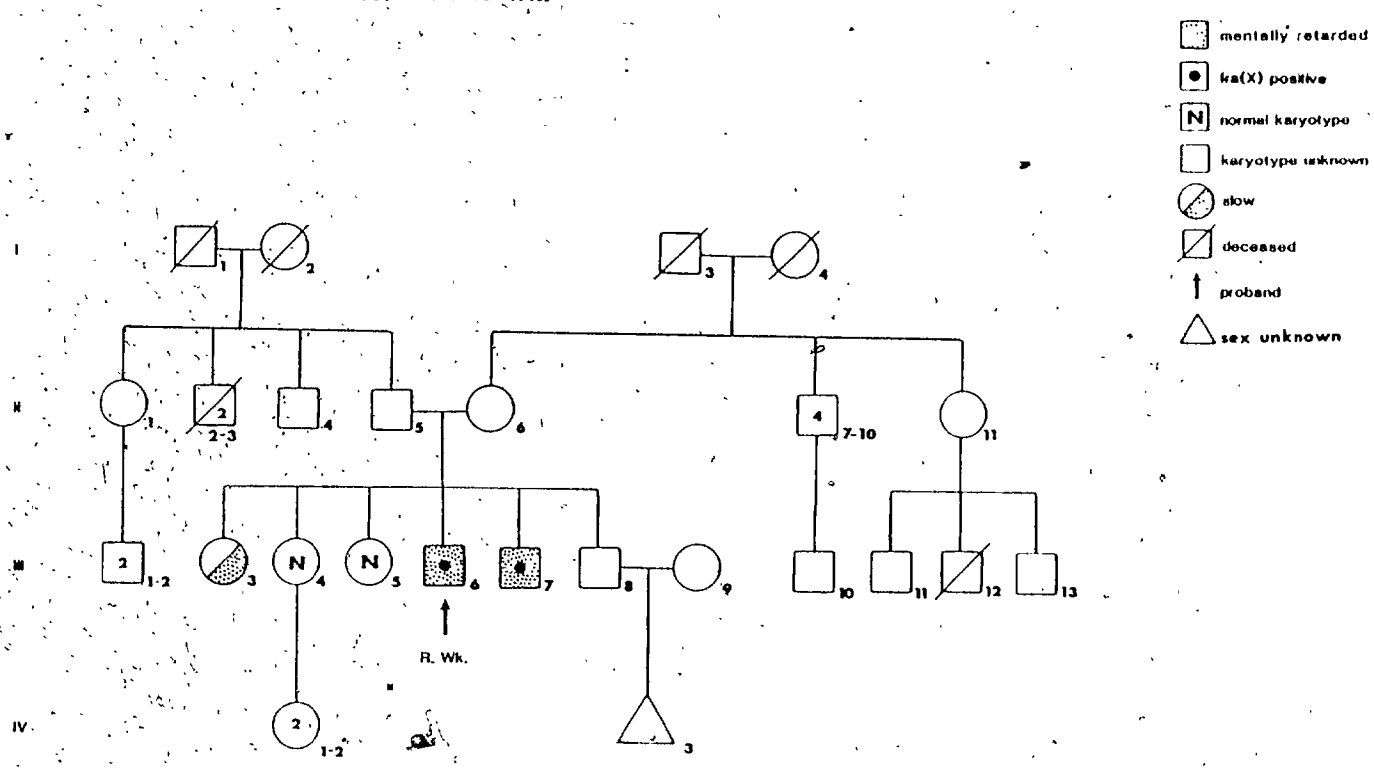
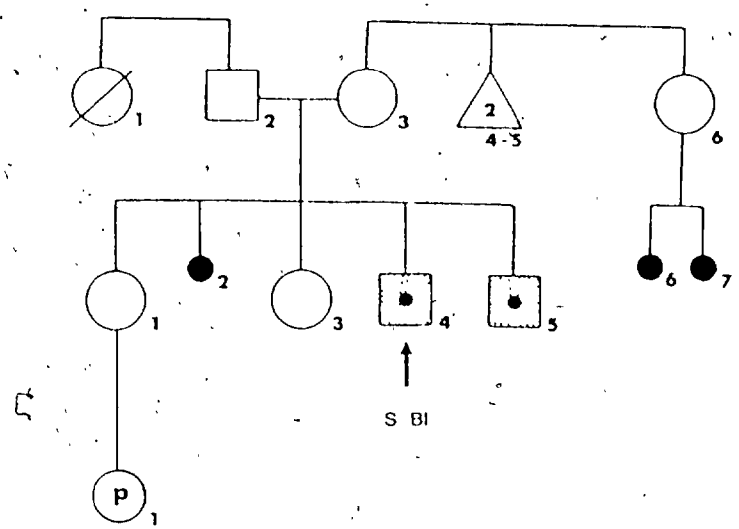


Figure 16. Pedigree of fra(X) family B1.

PEDIGREE OF FRA(X) FAMILY BI.



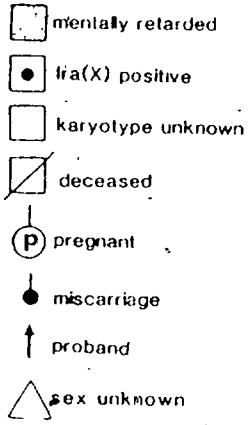
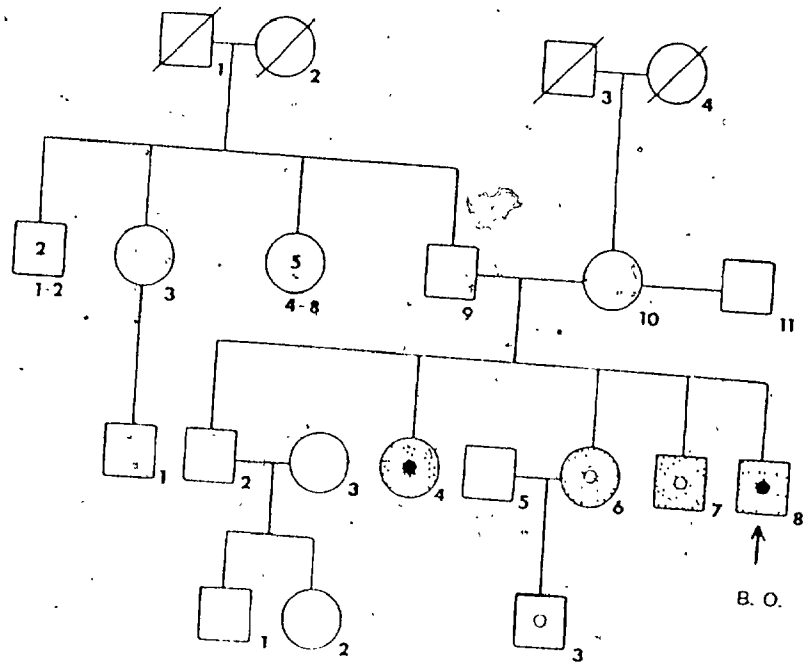


Figure 17. Pedigree of fra(X) family O.

PEDIGREE OF FRA(X) FAMILY O.



mentally retarded
fra(X) positive
not tested for fra(X)
karyotype unknown
deceased
proband

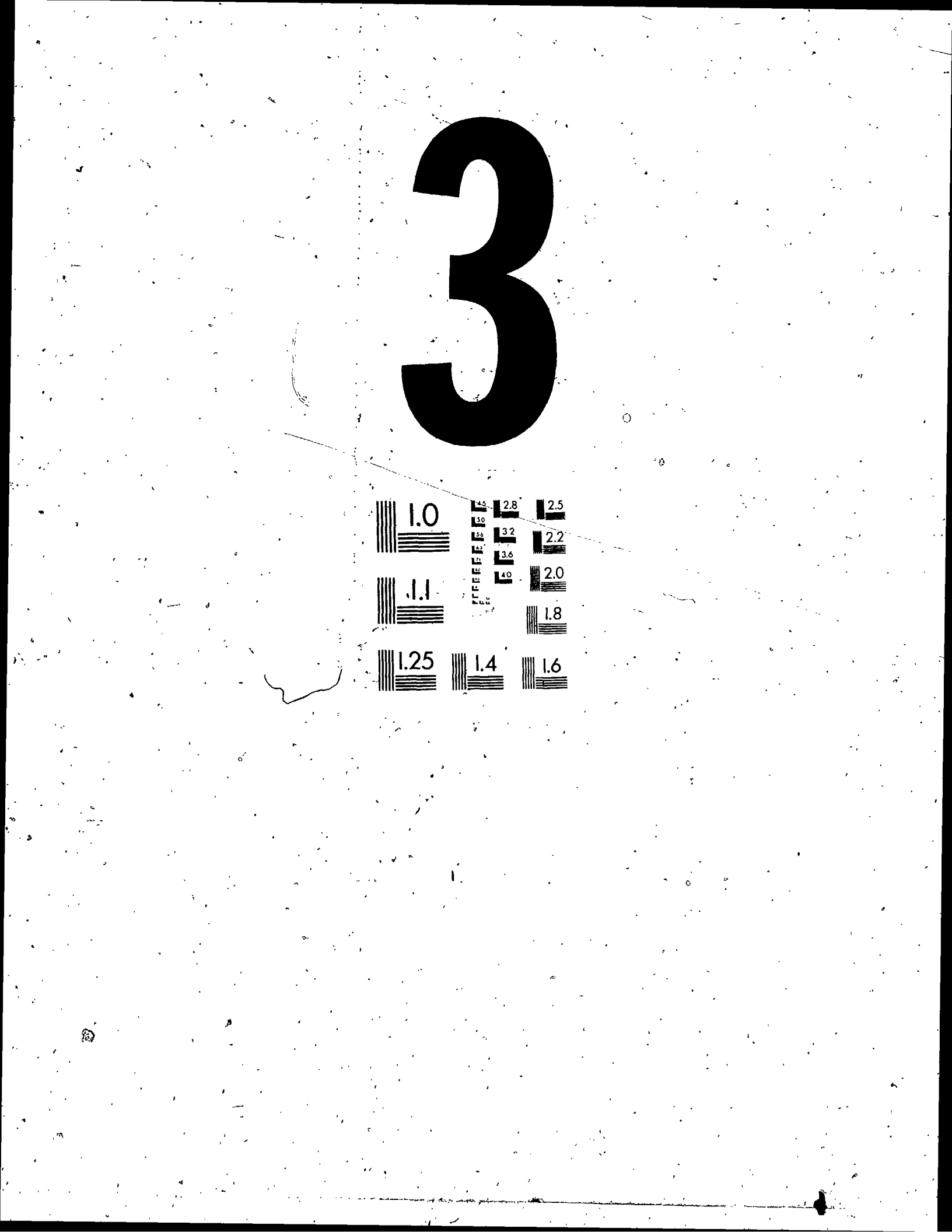

3
1.0
1.1
1.25
1.4
1.6
2.8
3.2
3.6
4.0
2.5
2.2
2.0
1.8

Figure 18. Pedigree of fra(X) family Bo.

PEDIGREE OF FRA(X) FAMILY Bo.

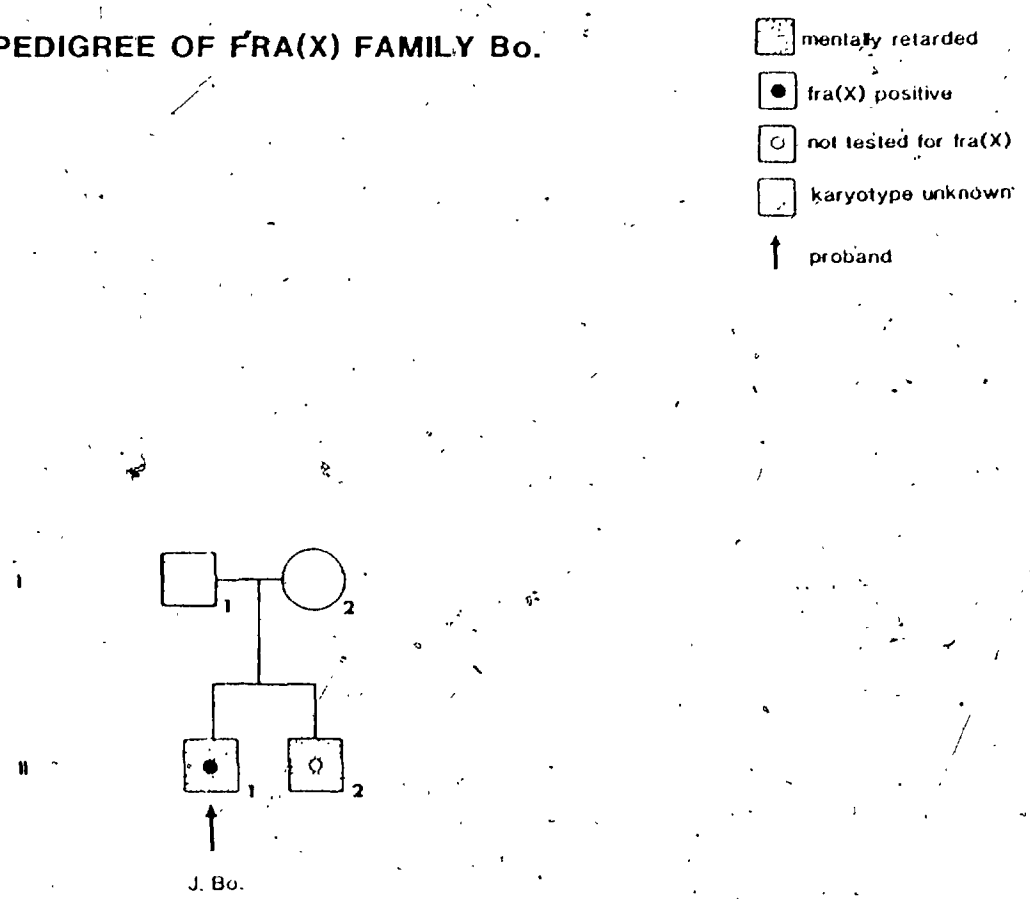


Figure 19. Pedigree of fra(X) family Mn.

PEDIGREE OF FRA(X) FAMILY Mn.

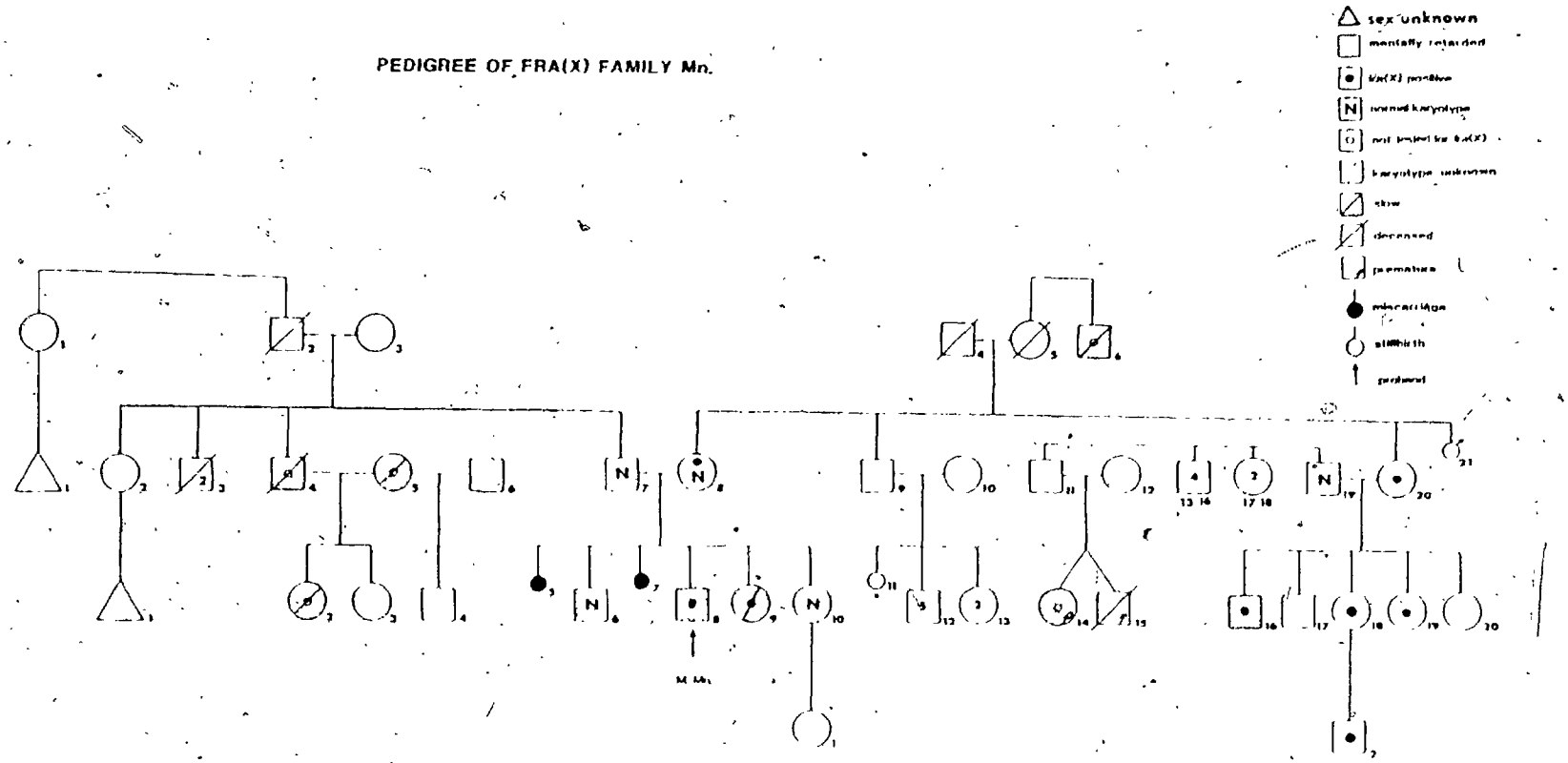


Figure 20. Pedigree of fra(X) family Mt.

PEDIGREE OF FRA(X) FAMILY Mt.

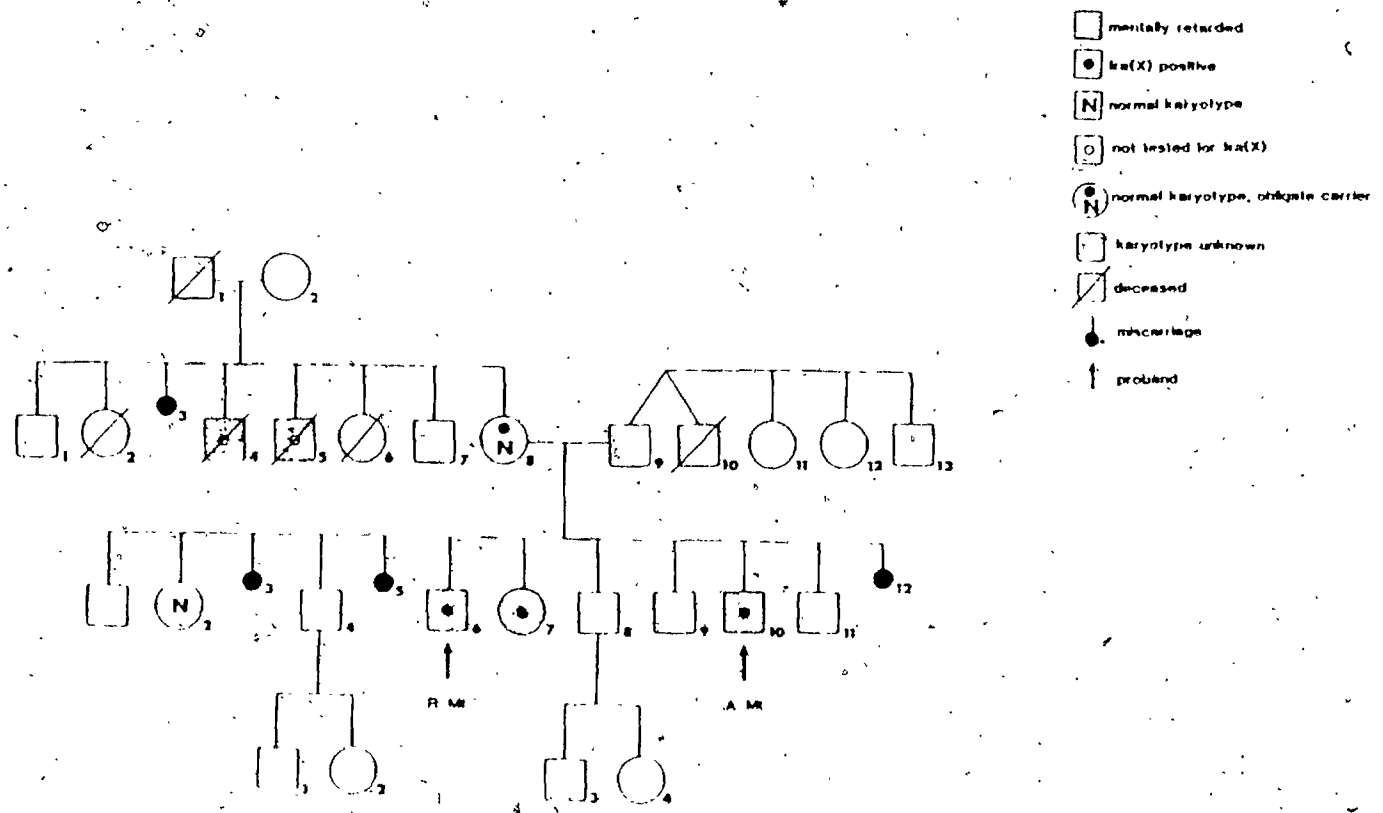


Figure 21. Pedigree of fra(X) family W-B.

PEDIGREE OF FRA(X) FAMILY W-8.

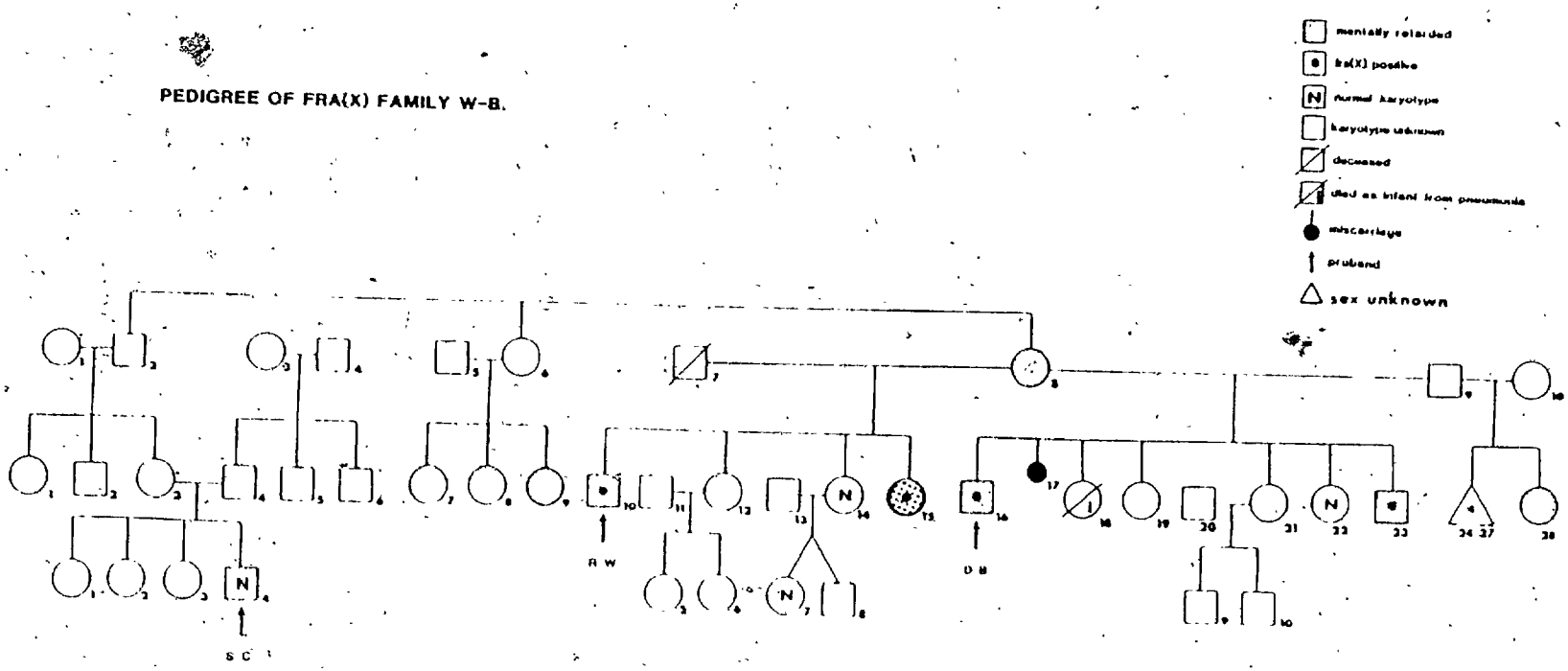


Figure 22. Pedigree of fra(X) family W.

PEDIGREE OF FRA(X) FAMILY W.

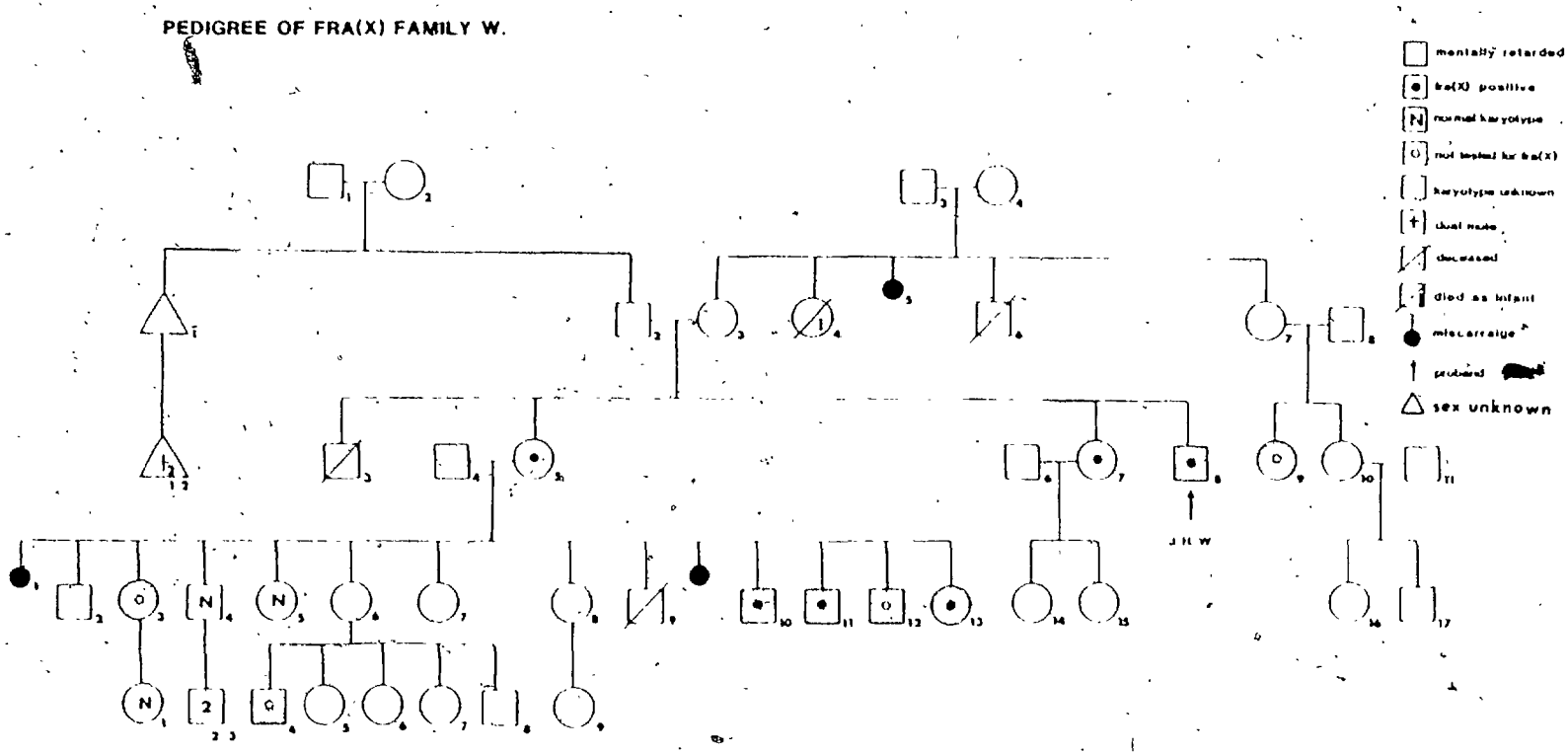
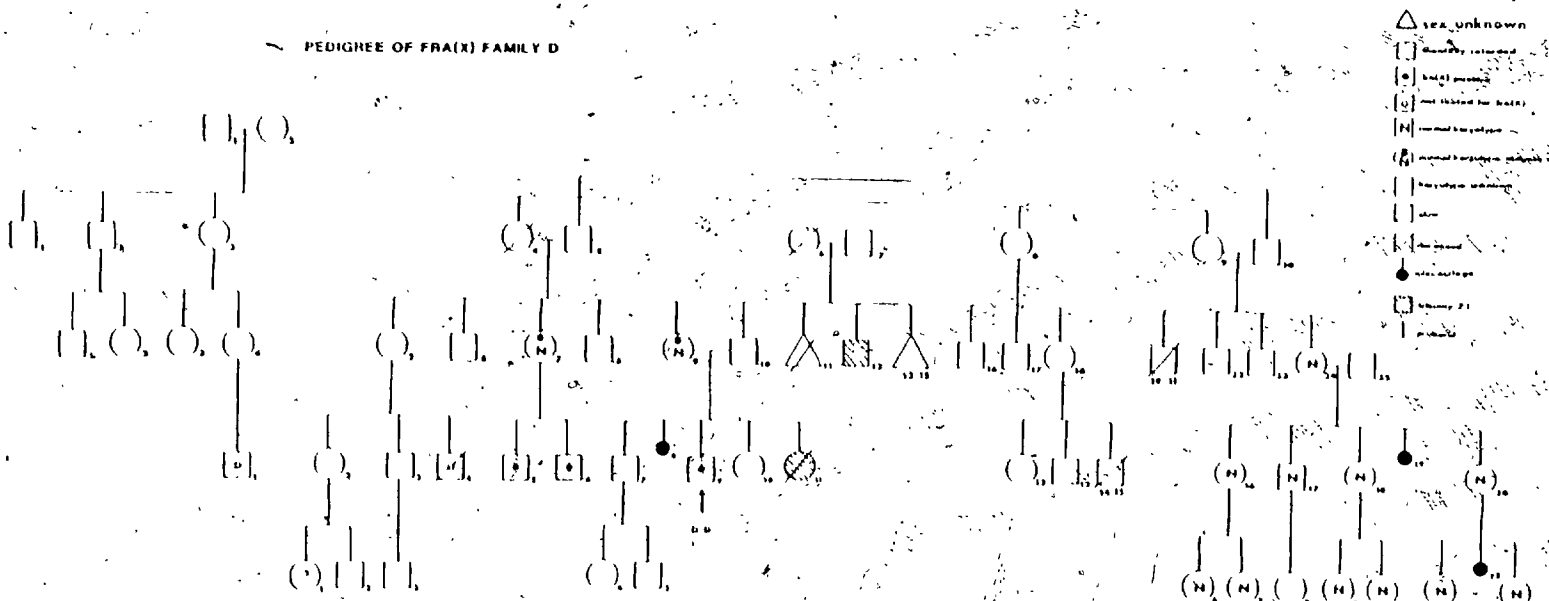


Figure 23. Pedigree of fra(X) family D.

PEDIGREE OF FRA(X) FAMILY D



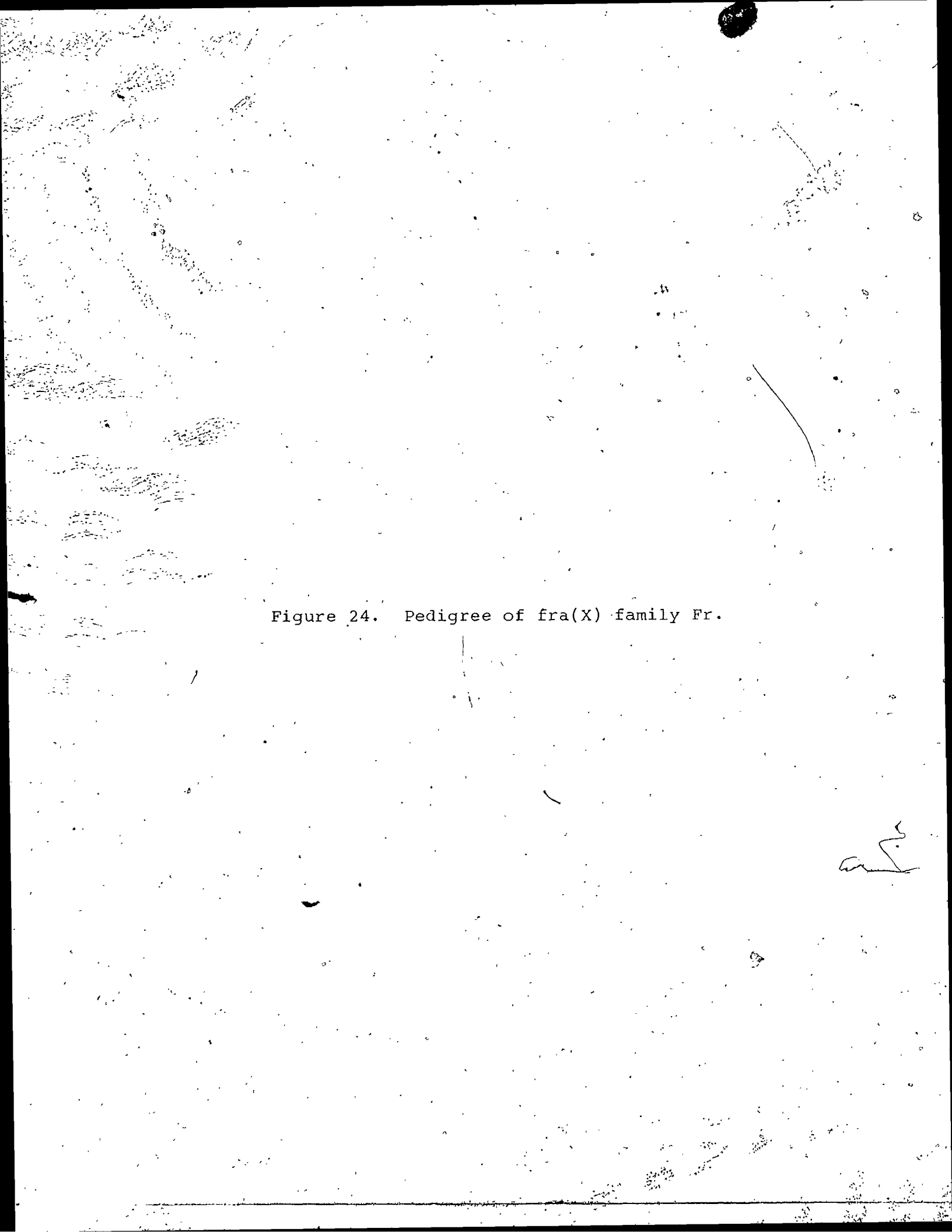


Figure 24. Pedigree of fra(X) family Fr.

PEDIGREE OF FRA(X) FAMILY Fr.

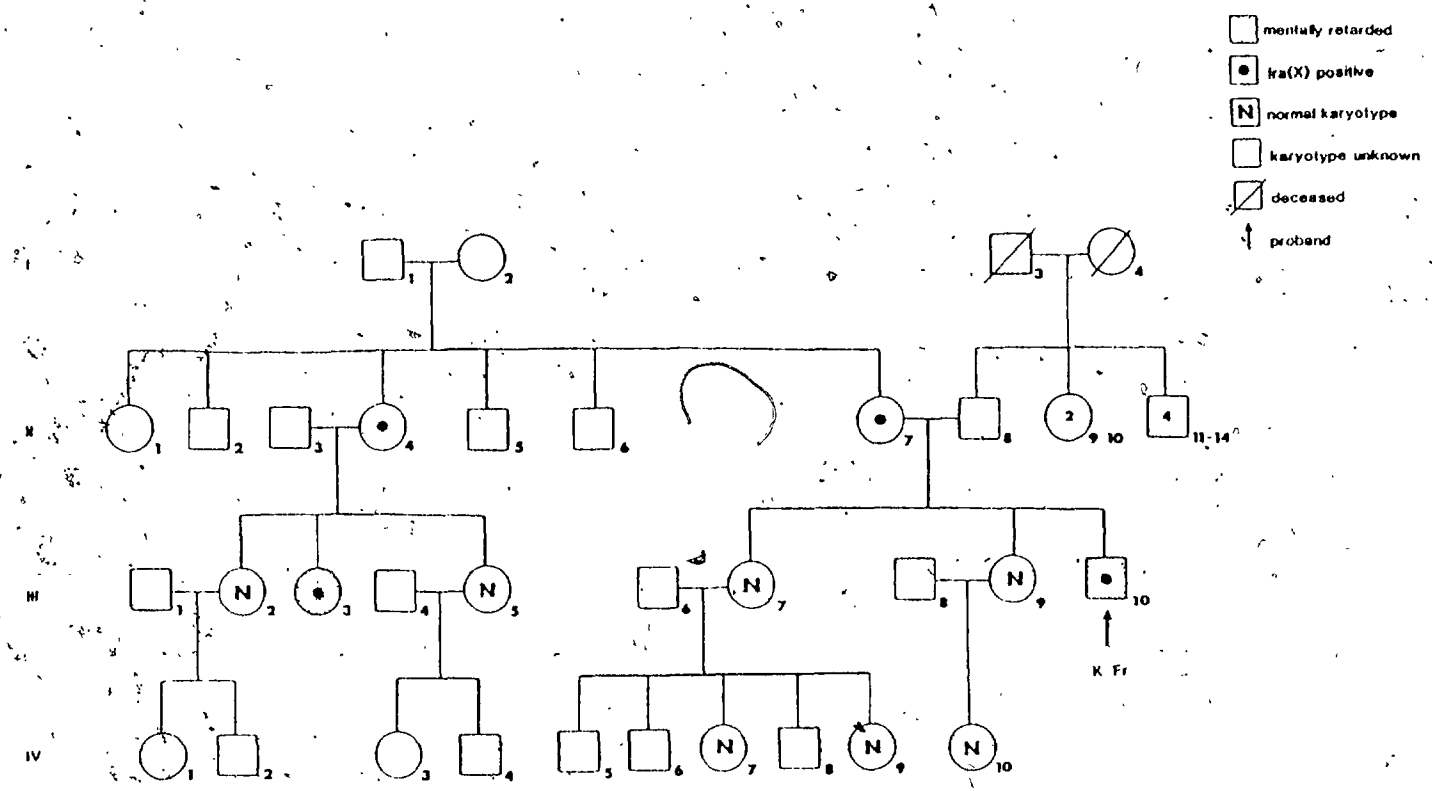


Figure 25. Pedigree of fra(X) family C.

PEDIGREE OF FRA(X) FAMILY C.

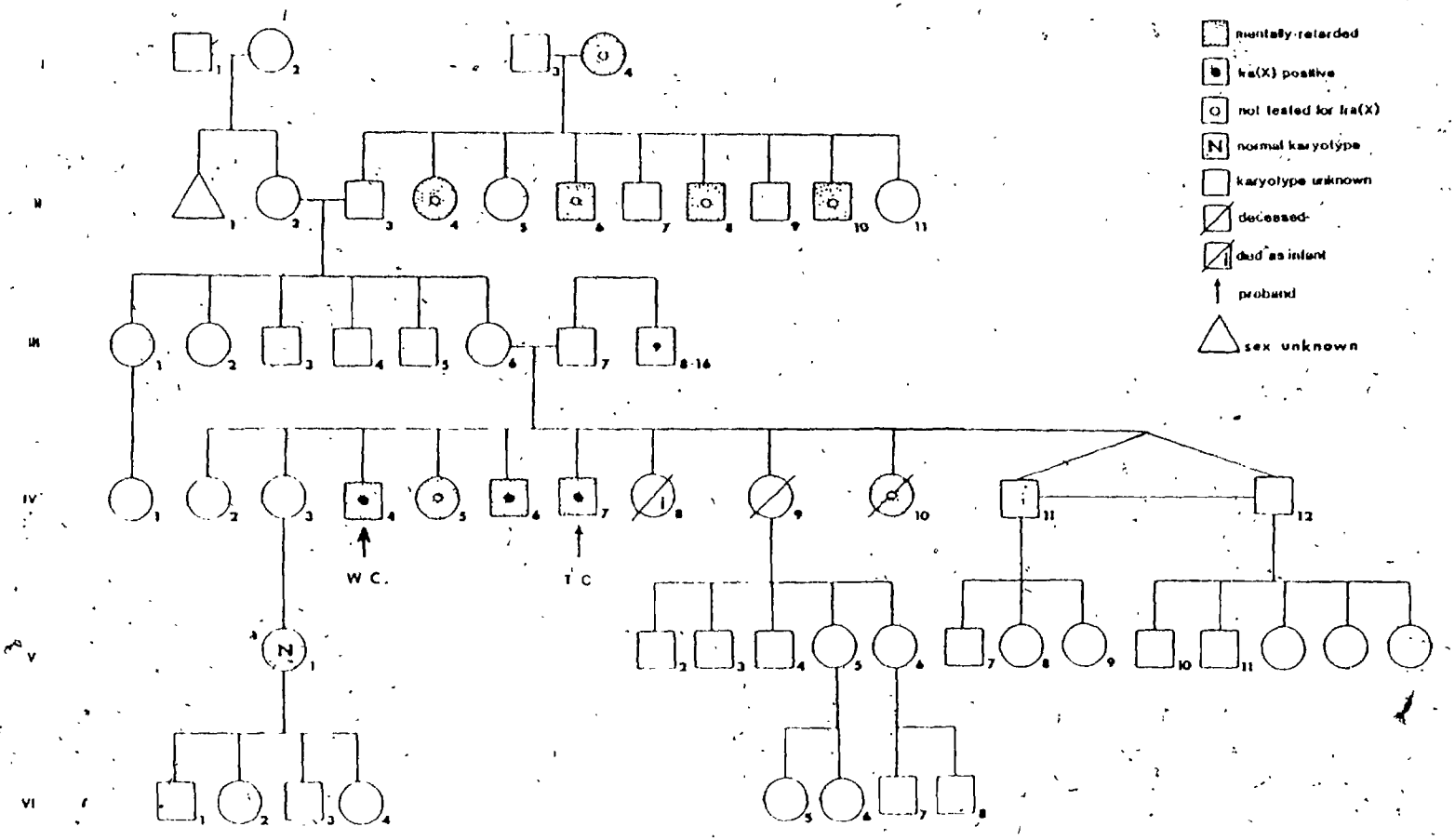


Figure 26. Pedigree of fra(X) family Bc.

PEDIGREE OF FRA(X) FAMILY B..

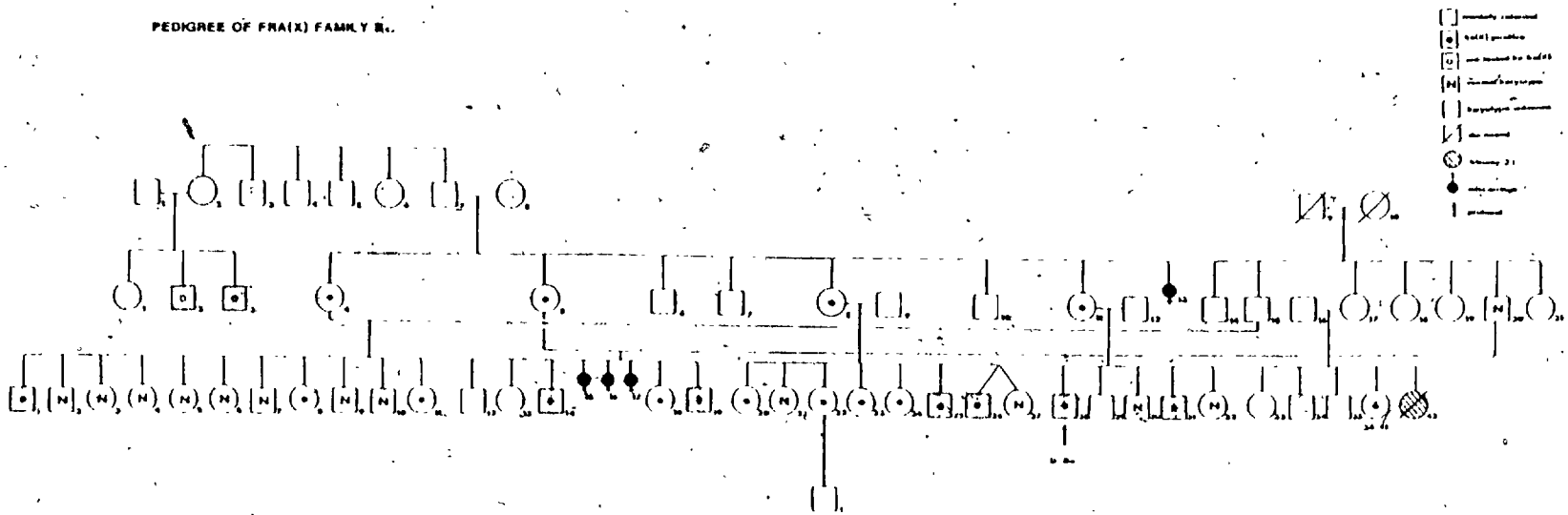
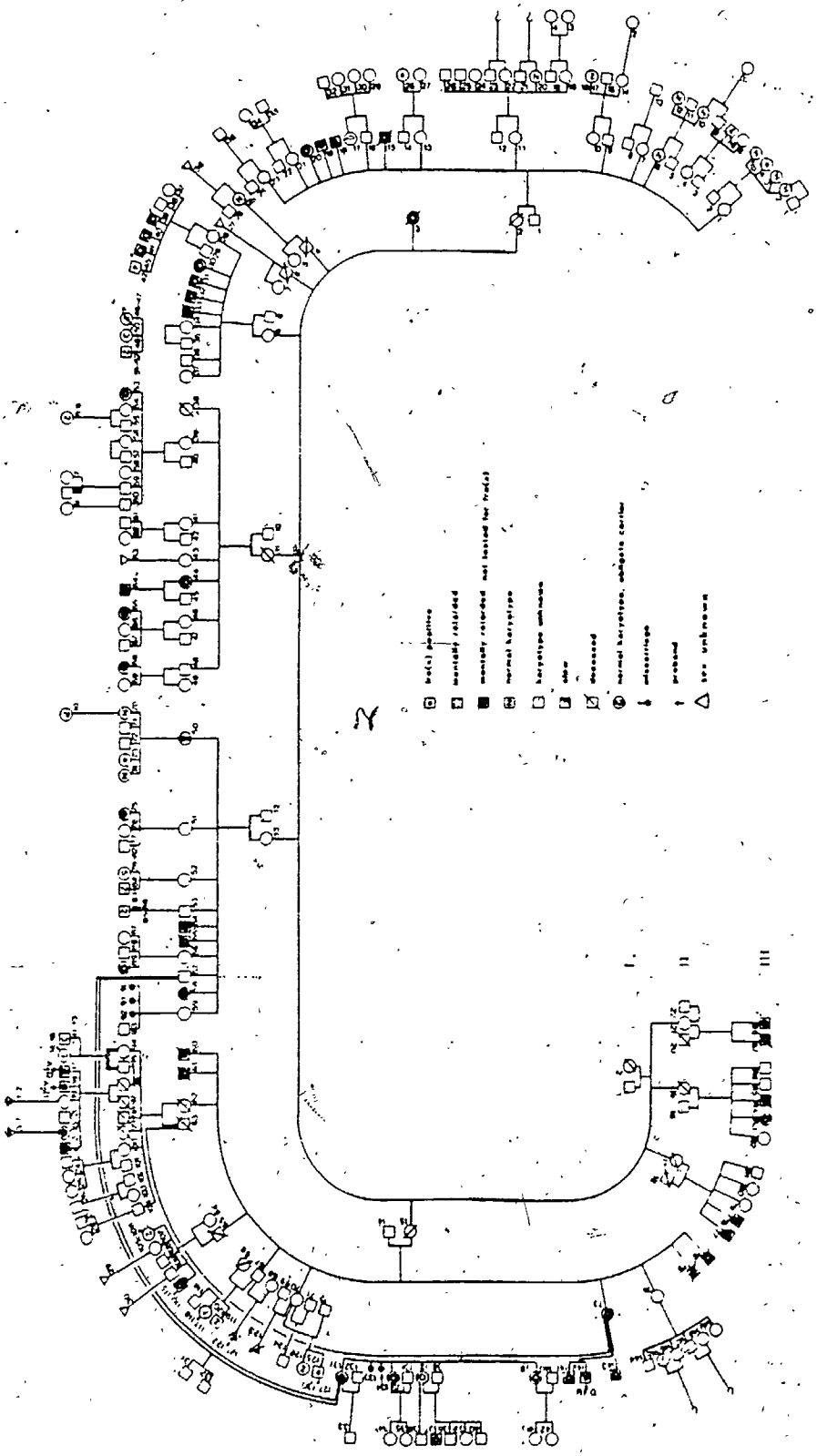


Figure 27. Pedigree of fra(X) family Fg.



PEDIGREE OF FRAGILE X FAMILY Fg.

Figure 28. Distribution of testicular volume (ml) versus age (yr) in fra(X) positive males.

⊙ , Negative family history of MR.

1. Family Bl
   a. Bl.
   b. S.Bl

2. Family W-B
   a. D.B.
   b. W.B.
   c. R.W.

3. Family C
   a. A.C.
   b. W.C.
   c. T.C.

4. Family D
   a. D.D.
   b. D.M.

5. Family Mt
   a. A.Mt.
   b. L.Mt.

6. Family Fg
   a. A_2D.
   b. A_1D.
   c. R.Fg.

7. Family O (B.O.)

8. Family Bo (J.Bo.)

9. Family W (J.H.W.)

10. Family Mn (M.Mn.)

11. Family Fr (K.Fr.)

12. Family Wk (R.Wk.)

13. Family Bc (D.Bc.)

14. Family K (J.K.)

15. Family R (D.R.)

16. Family L (M.L.)

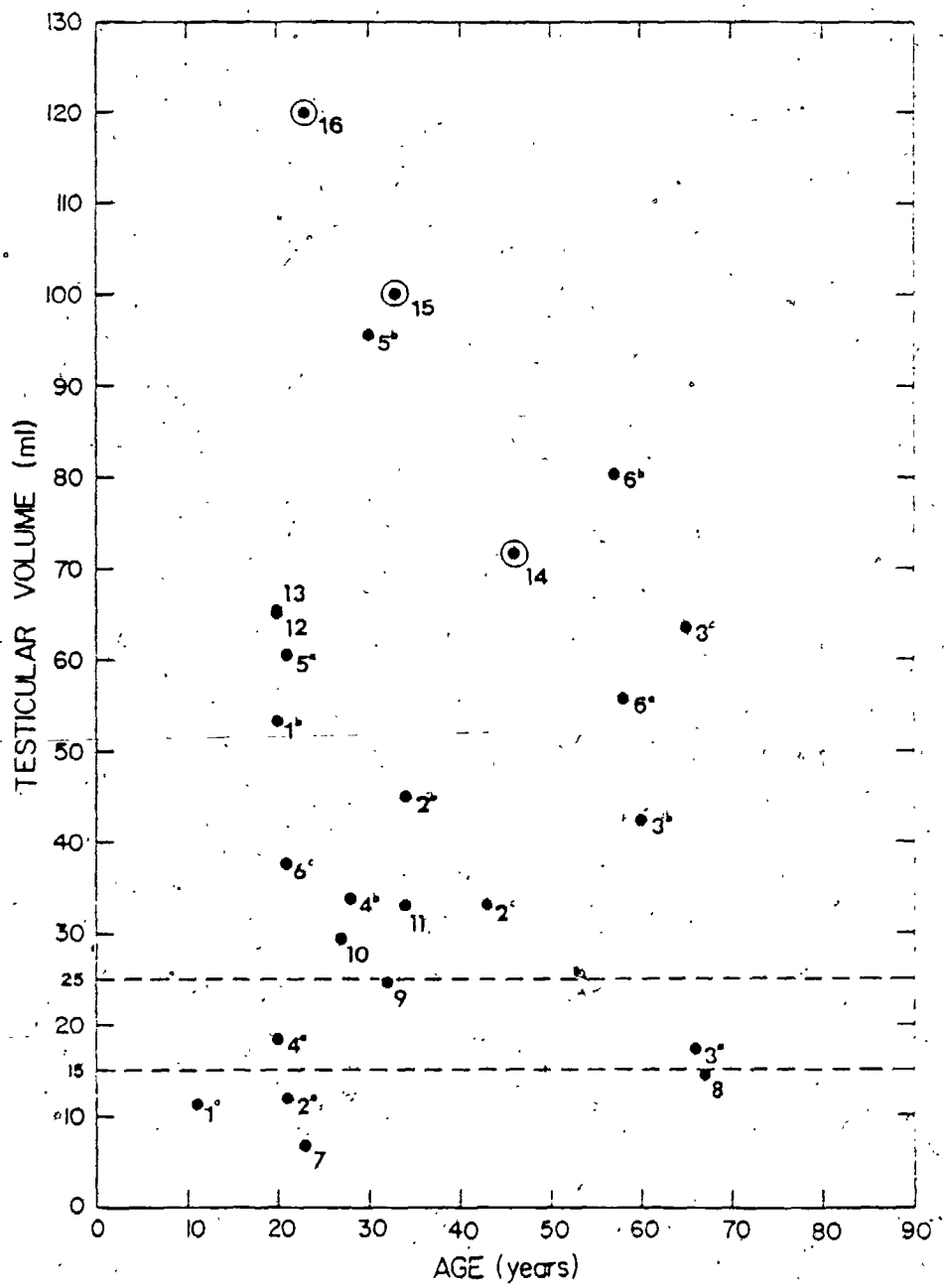

TESTICULAR VOLUME (ml)
AGE (years)
0
10
15
20
25
30
40
50
60
70
80
90
100
110
120
130
16
15
14
13
12
11
10
9
8
7
6
5
4
3
2
1

Figure 29. A comparison of the distribution of testicular volume (ml) in the fra(X) syndrome and other non-specific X-linked MR.

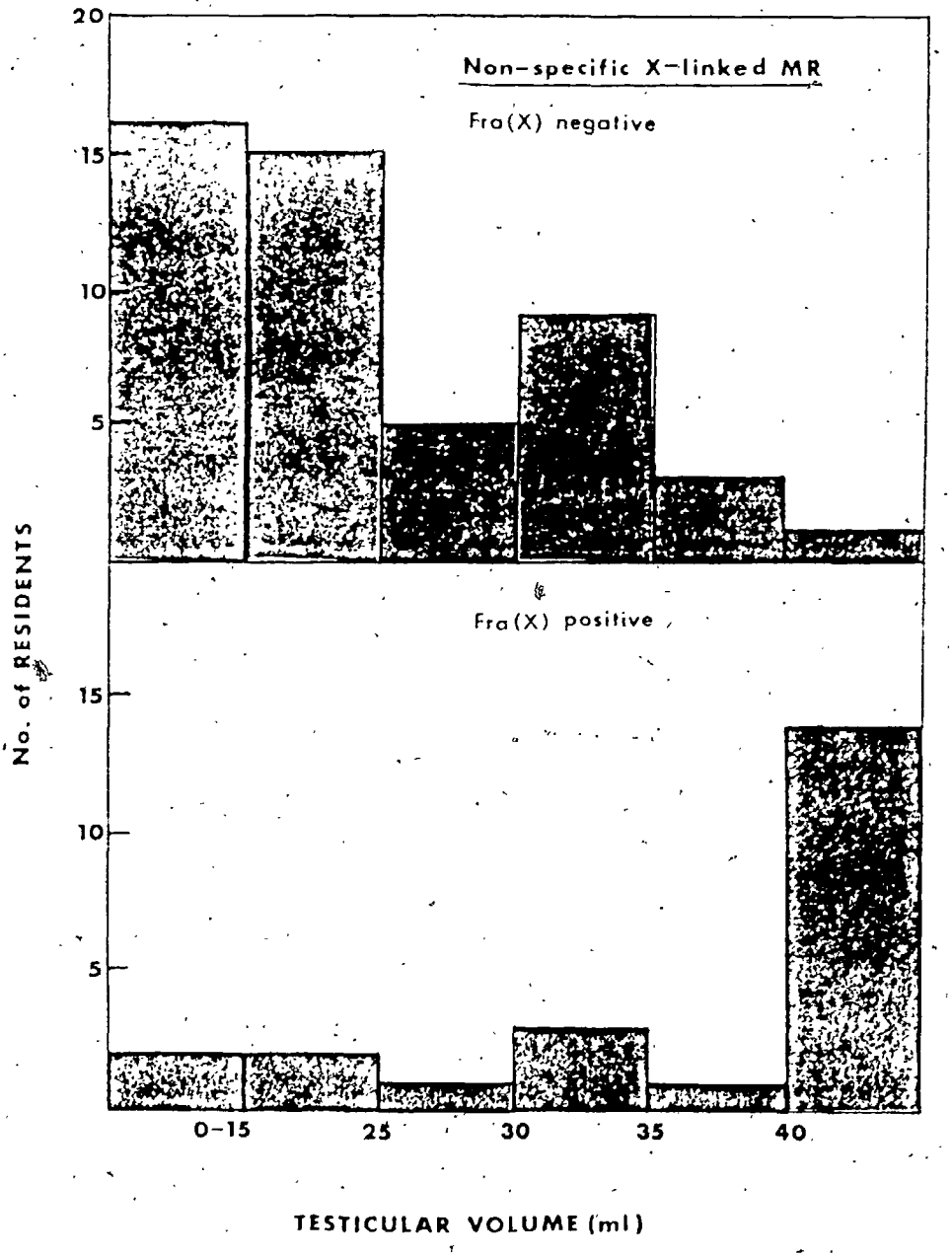
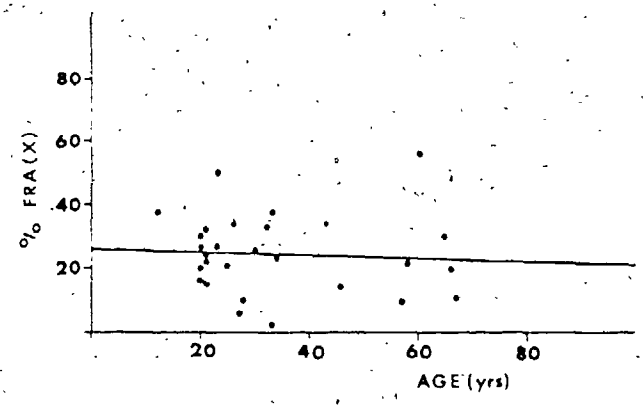

Non-specific X-linked MR
Fra(X) negative
Fra(X) positive
20
15
10
5
15
10
5
No. of RESIDENTS
0-15
25
30
35
40
TESTICULAR VOLUME (ml)

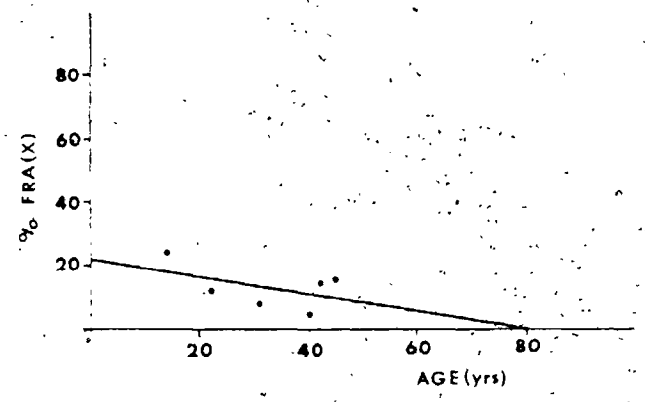
Figure 30. Linear regression analysis of percentage of fra(X) expression versus age (yr).

a. Hemizygotes
b. Affected heterozygotes
c. Non-affected heterozygotes

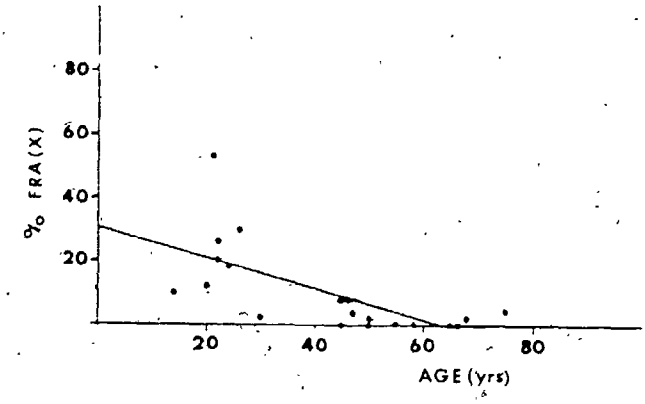
a)


80
60
40
20
% FRA(X)
20
40
60
80
AGE (yrs)

b)


80
60
40
20
% FRA(X)
20
40
60
80
AGE (yrs)

c)


80
60
40
20
% FRA(X)
20
40
60
80
AGE (yrs)

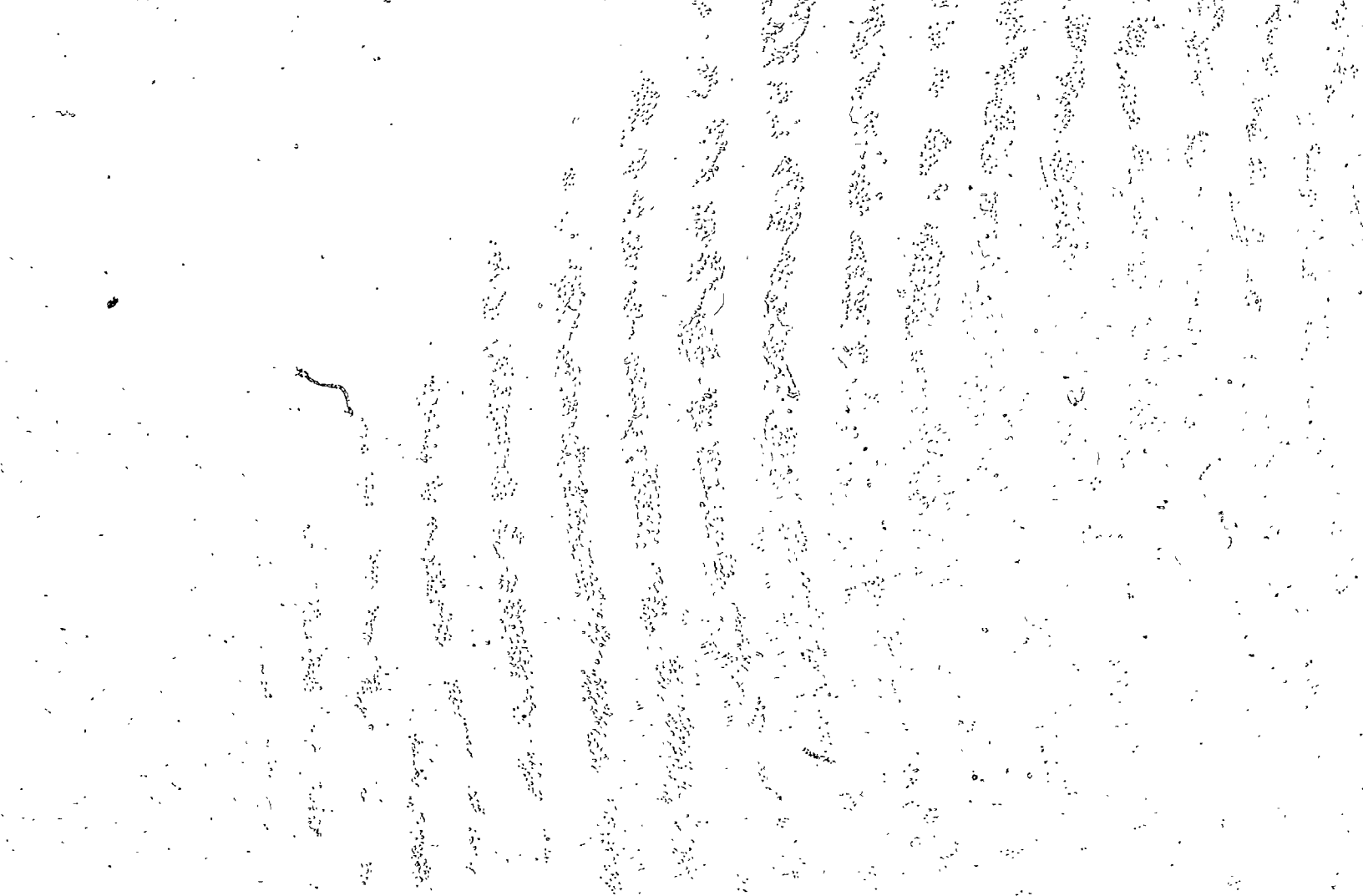
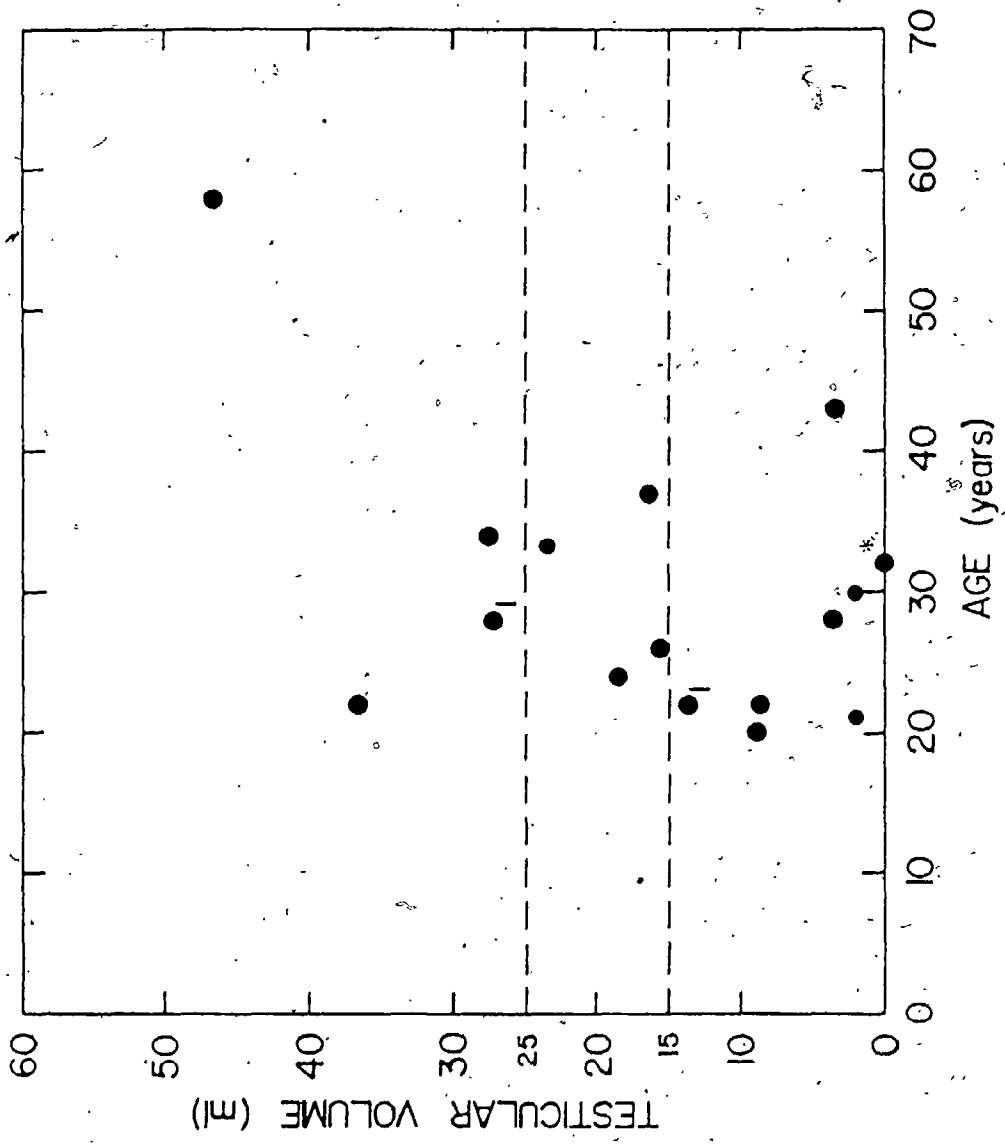


Figure 31. Distribution of testicular volume (ml) versus age (yr) in patients with chromosome abnormalities.

* cryptorchidism


TESTICULAR VOLUME (ml)
60
50
40
30
25
20
15
10
0
AGE (years)
0
10
20
30
40
50
60
70

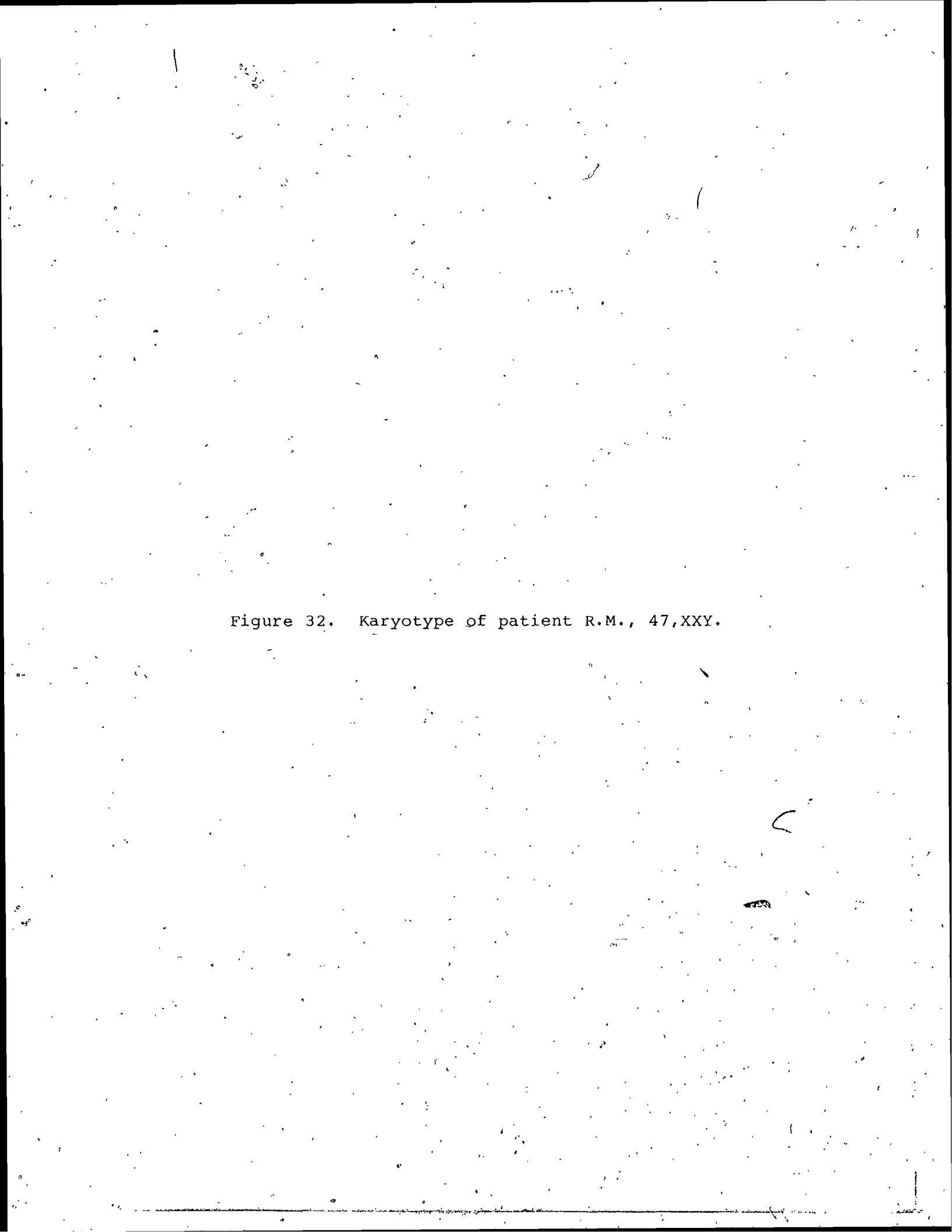


Figure 32. Karyotype of patient R.M., 47,XXY.

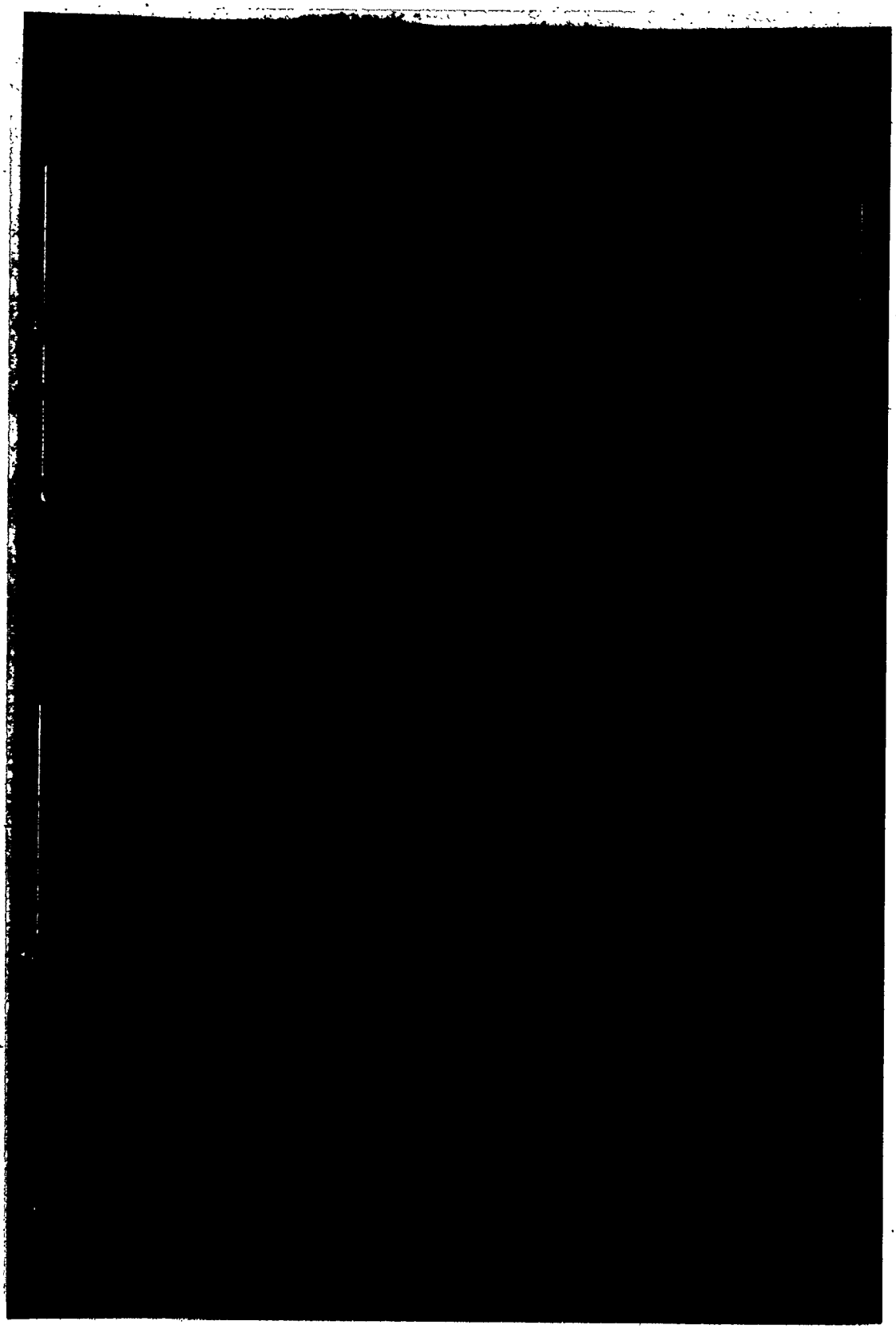
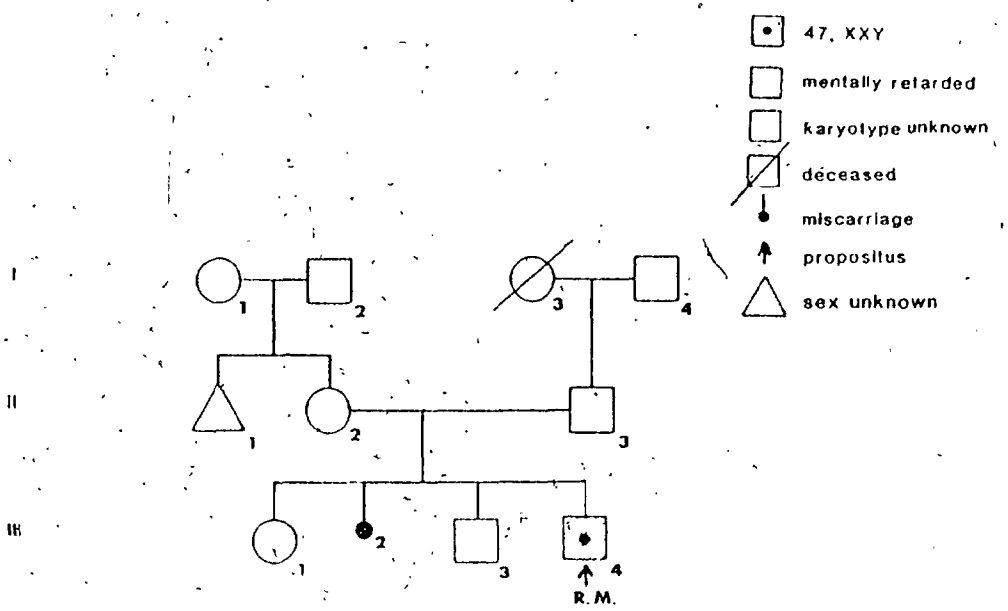


Figure 33. Pedigree of a family M with a 47,XXY

PEDIGREE OF A FAMILY WITH A 47,XXY



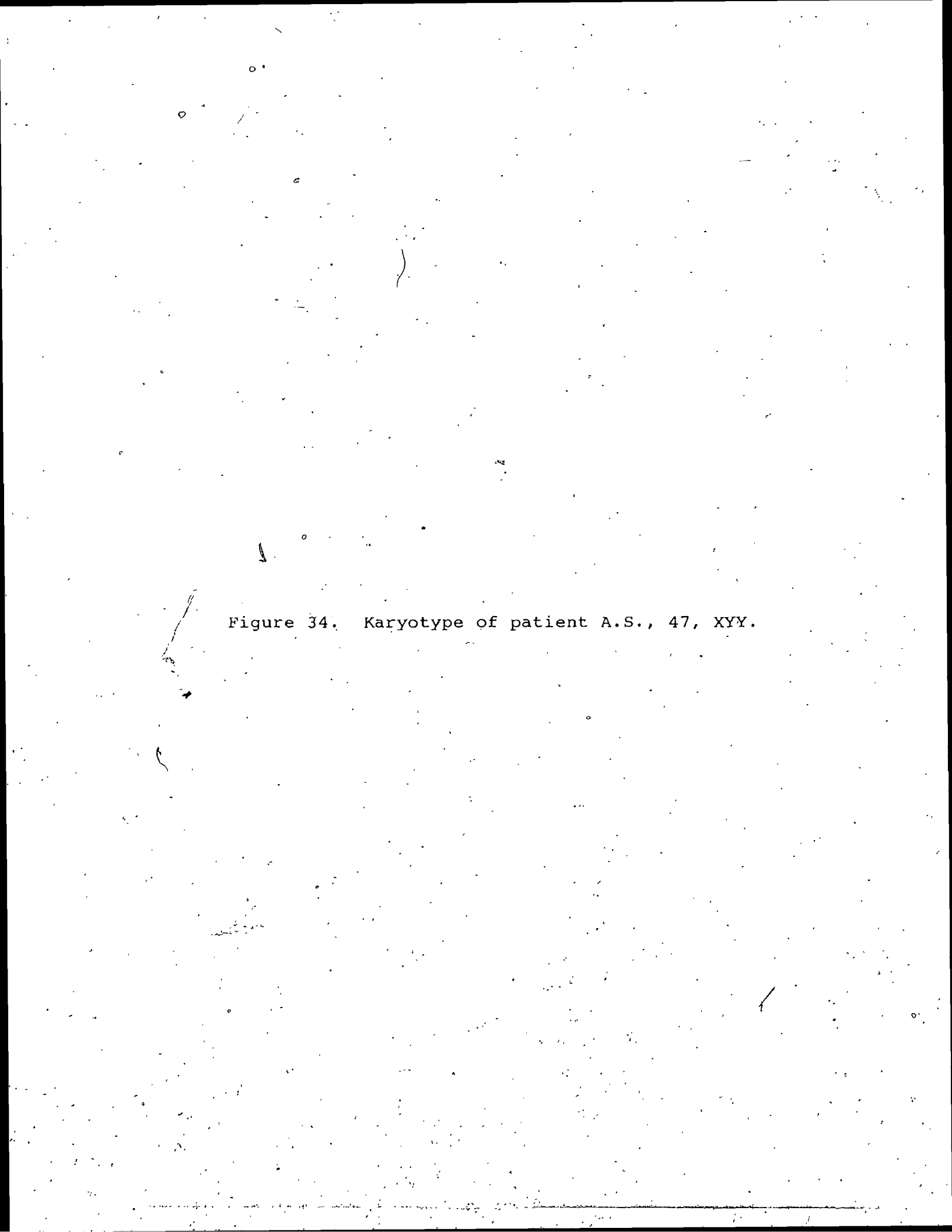


Figure 34. Karyotype of patient A.S., 47, XYY.

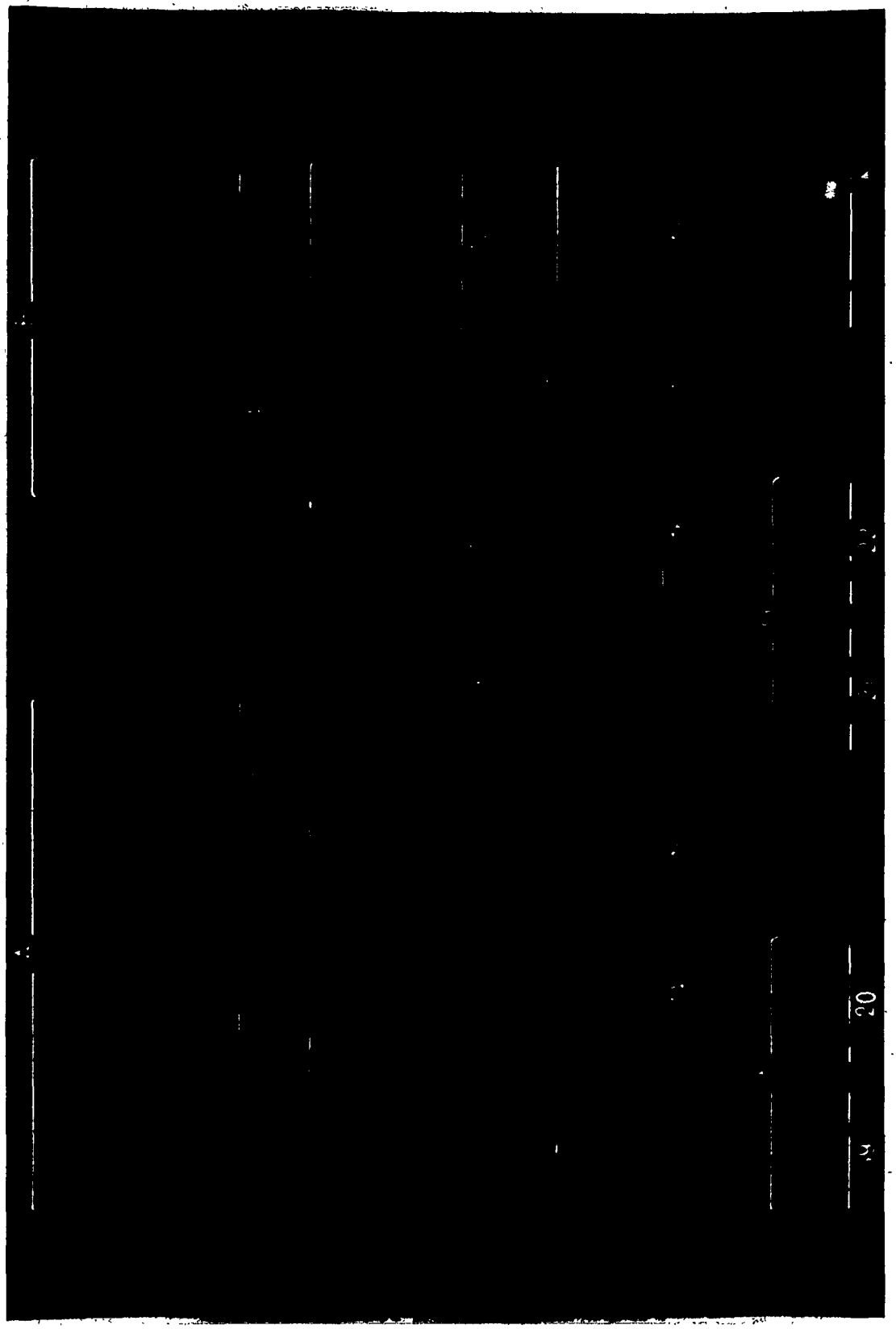

20

Figure 35. Pedigree of a family S with a 47,XYY

PEDIGREE OF THE FAMILY WITH A 47, XYY

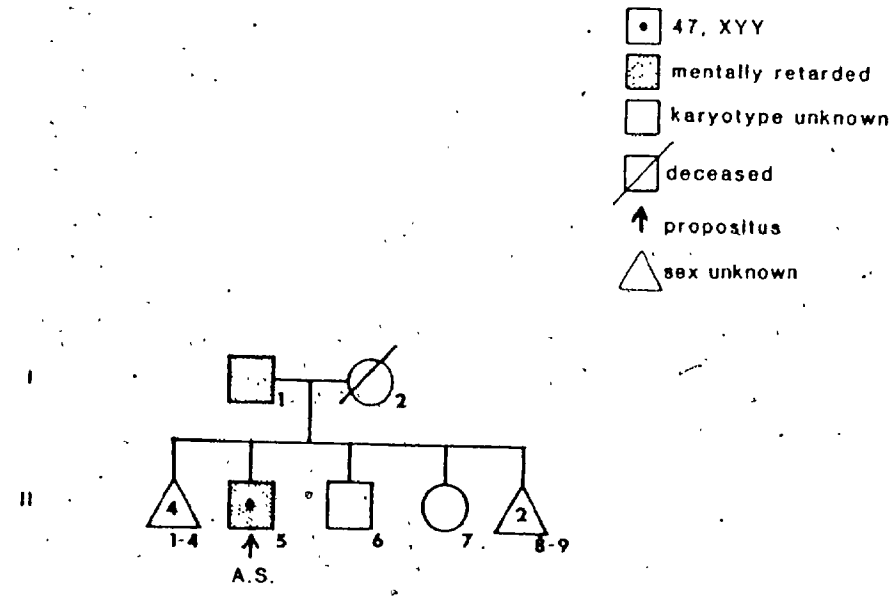


Figure 36. Karyotype of patient M.K., 46,XY,del(5)(p14 → pter)

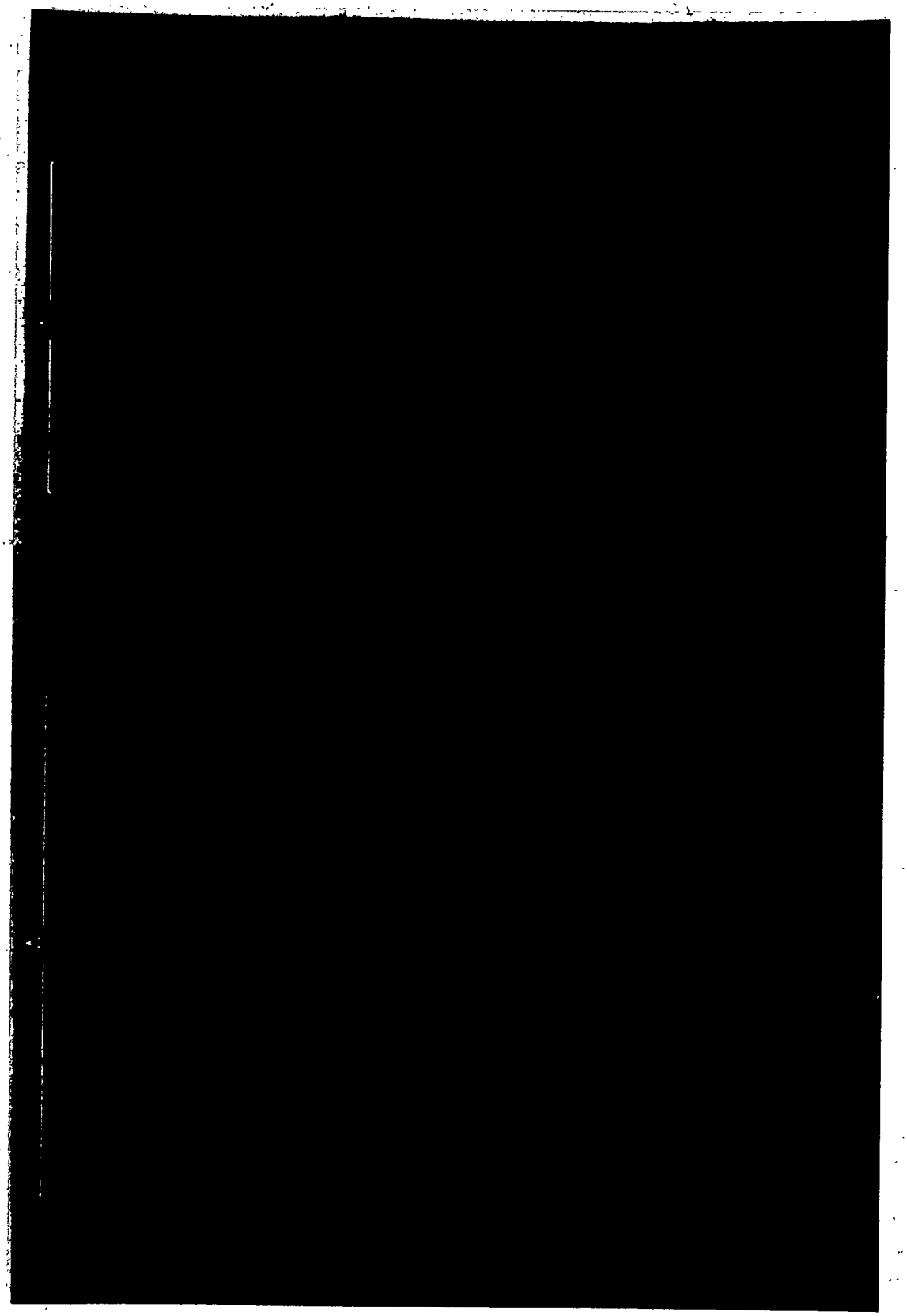


Figure 37. Pedigree of the family K with a 46,XY, del(5)(p14→pter)

PEDIGREE OF FAMILY K WITH A 46, XY, 5p-

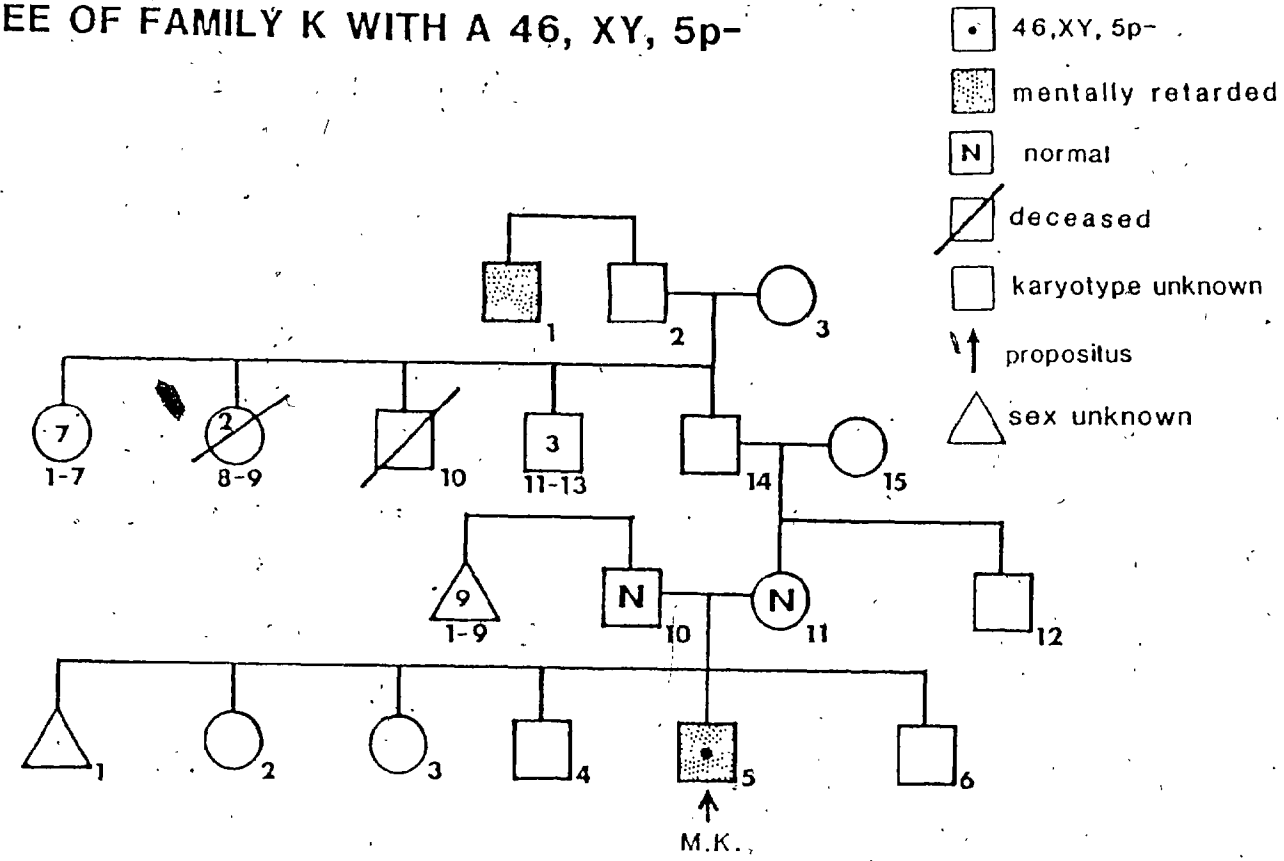
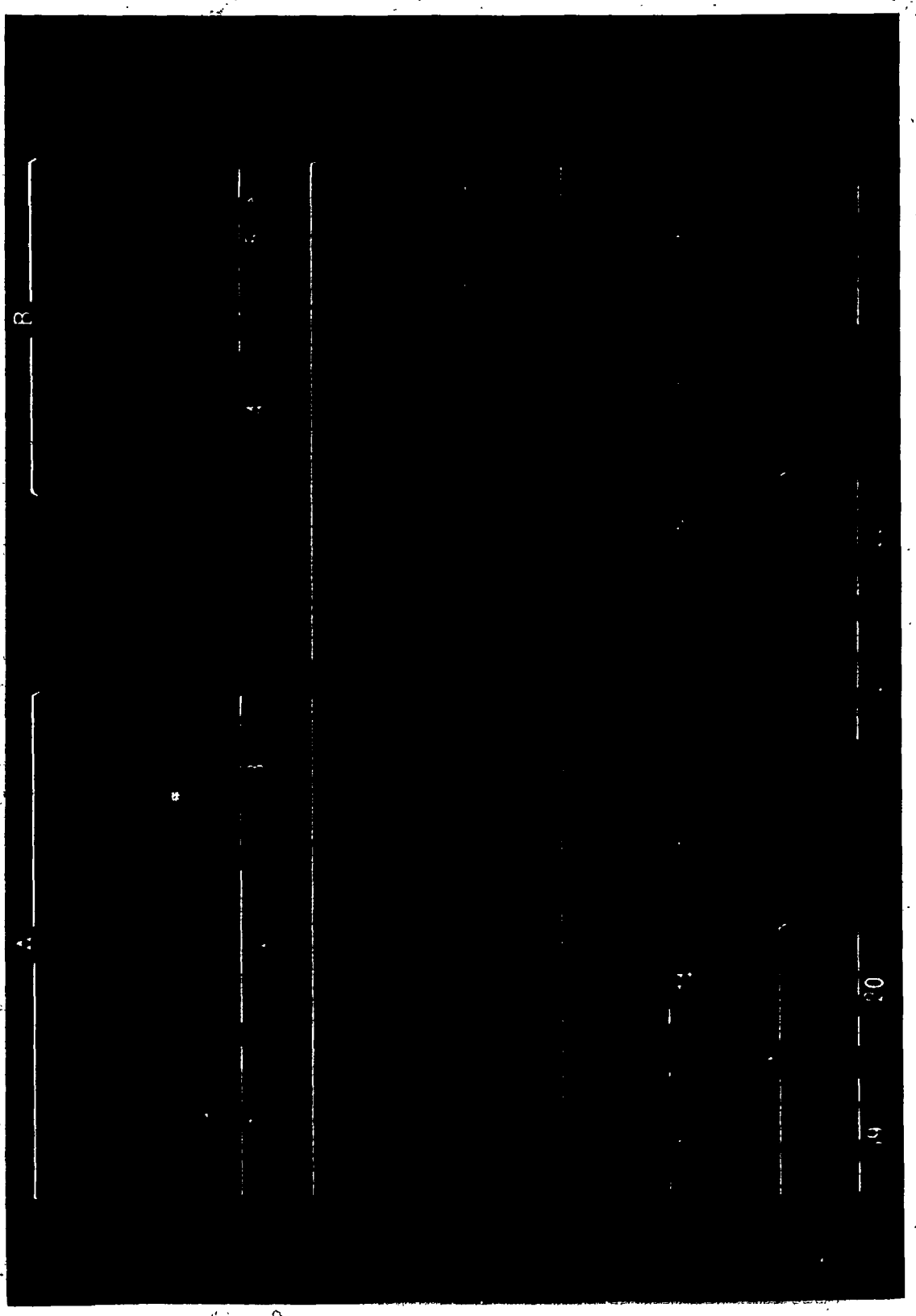


Figure 38. Karyotype of patient M.Bg., 46,XY,del(5)(p14→pter)


B
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20

Figure 39. Pedigree of family Bg with a 46,XY,del(5)(p14→pter)

PEDIGREE OF FAMILY Bg. WITH A 46, XY, 5p-

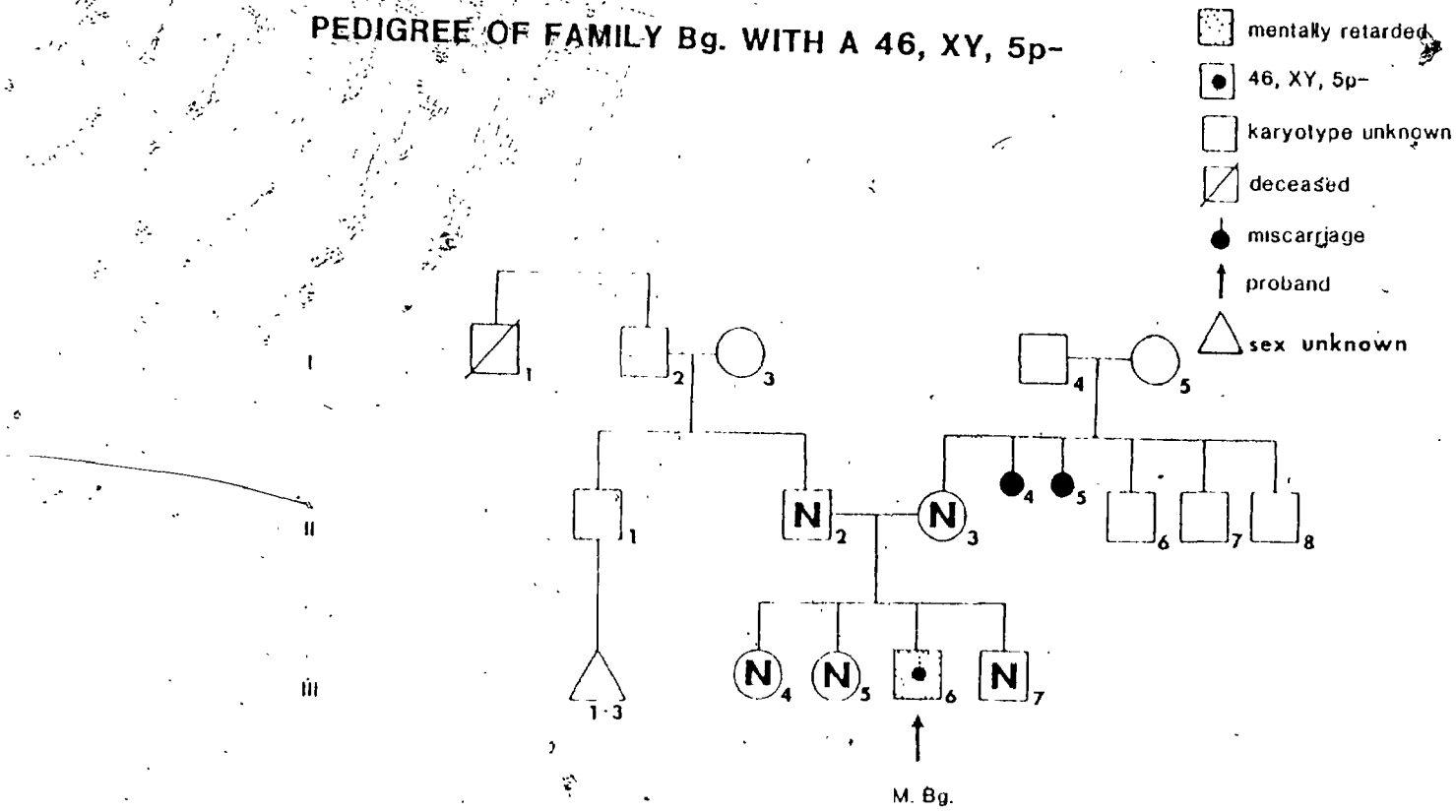

Figure 40. Karyotype of patient G.B., 46,XY,del(5)(p14→pter)

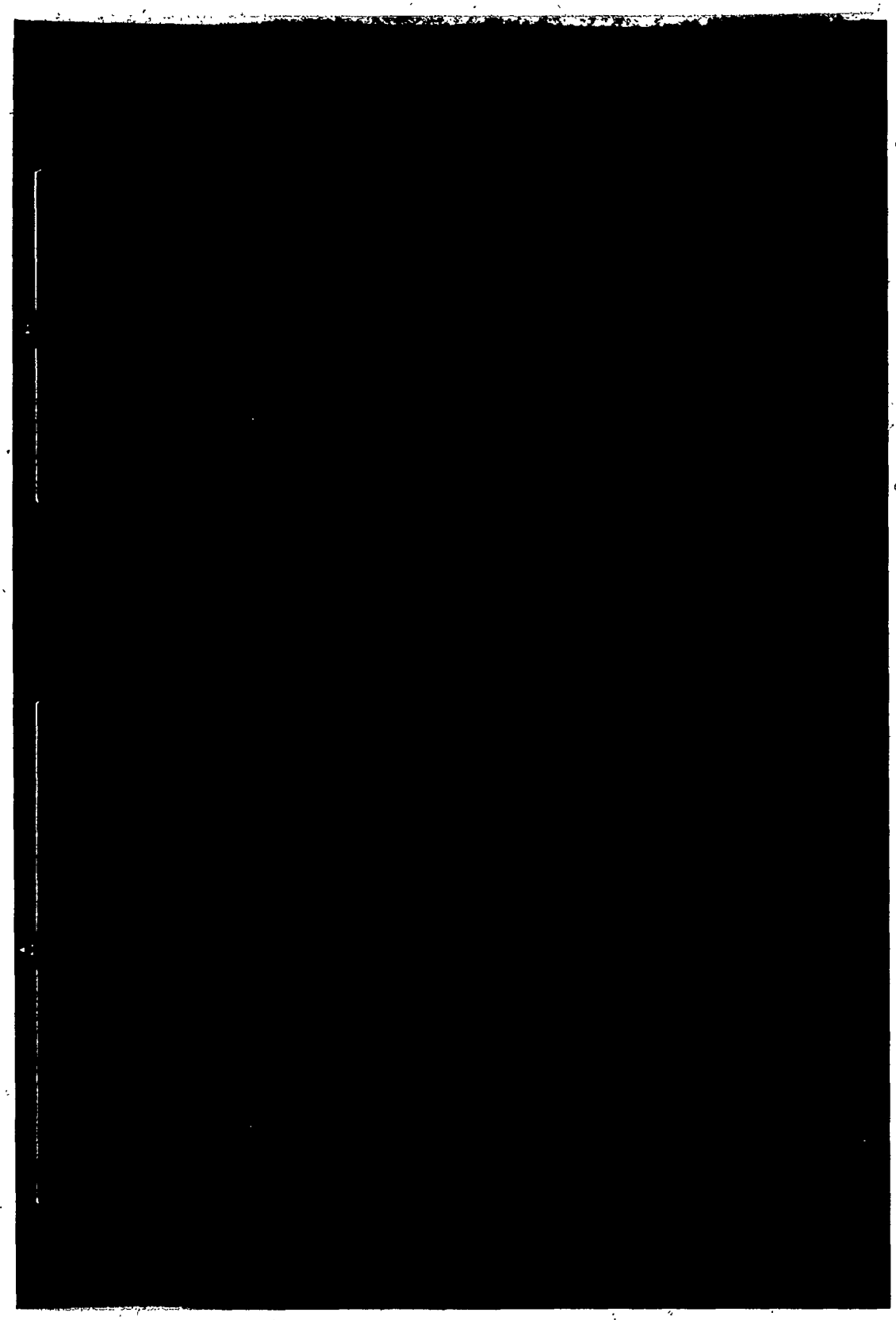

Figure 41. Pedigree of family B with a 46,XY,del(5)(p14→pter)

PEDIGREE OF FAMILY B WITH A 46, XY, 5p-

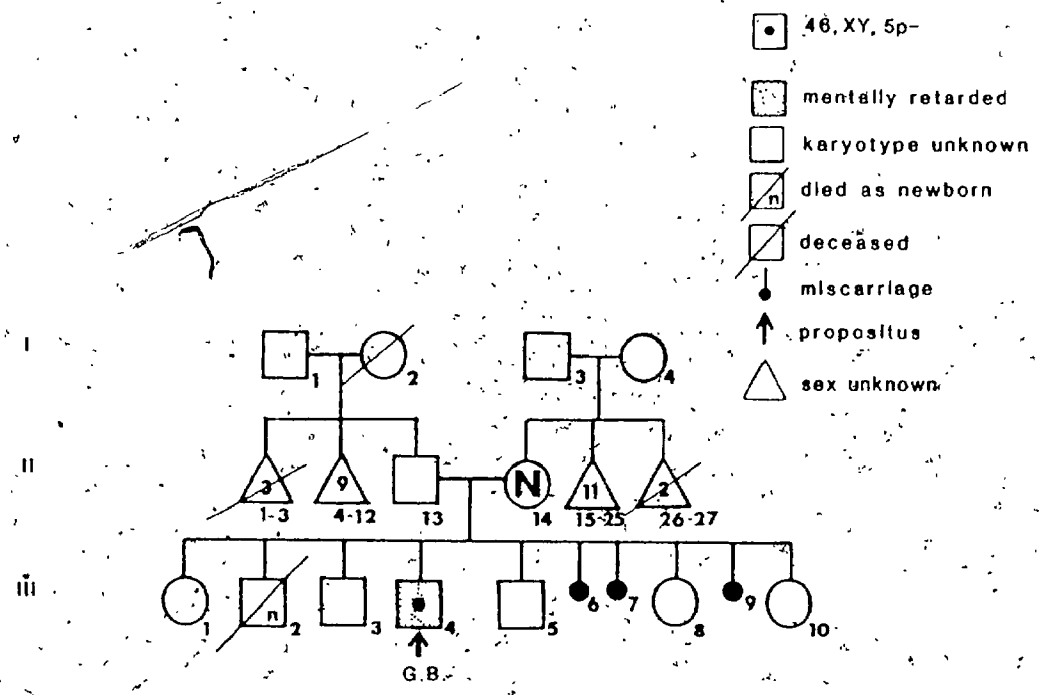

Figure 42. Karyotype of patient R.K., 45,XY,-13,-14, +t(13q14q)

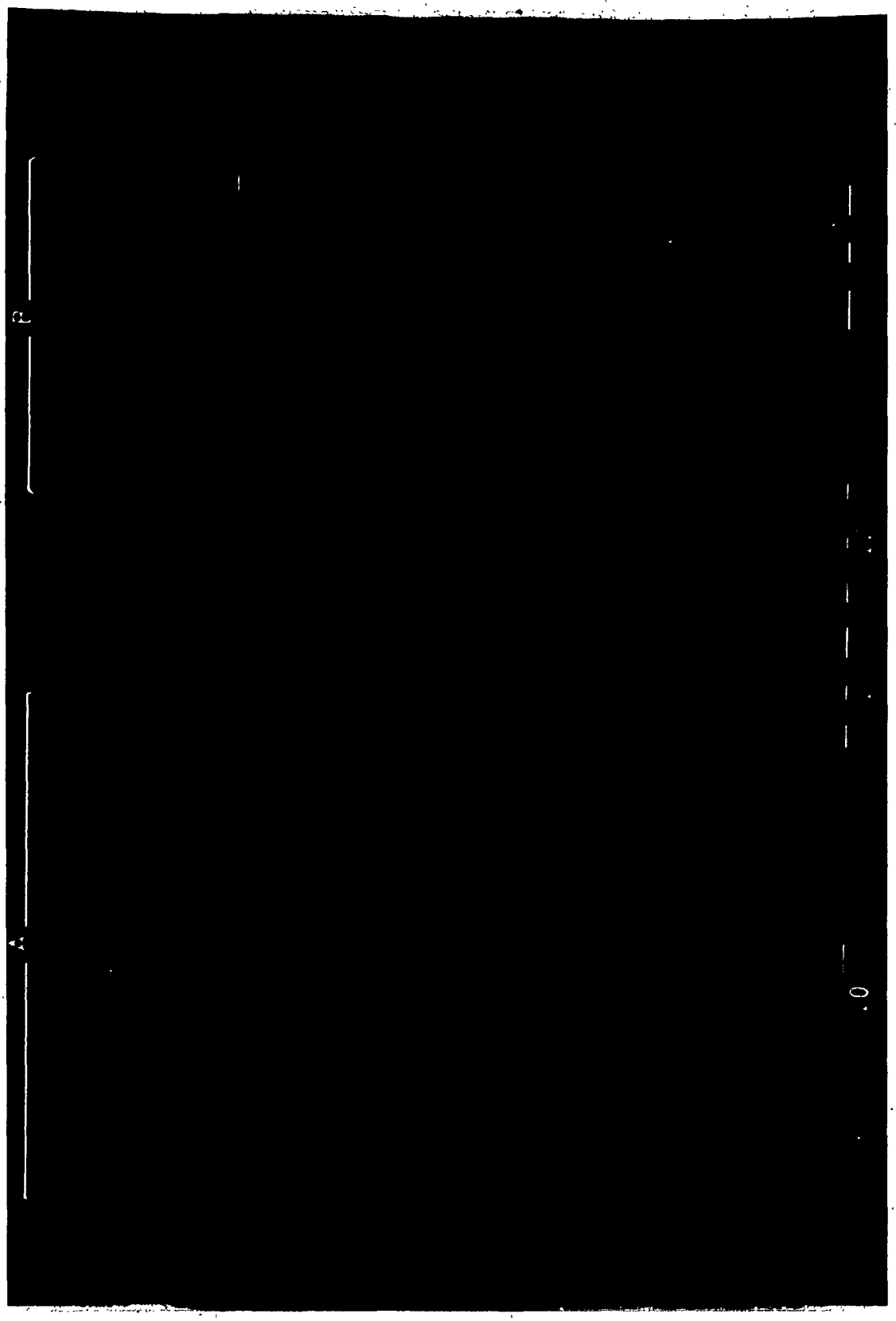

Figure 43. Pedigree of the family K with 45,XY,-13,-14,
+t(13q14q)

PEDIGREE OF THE FAMILY WITH 45, XY, -13, -14,+t(13q14q)

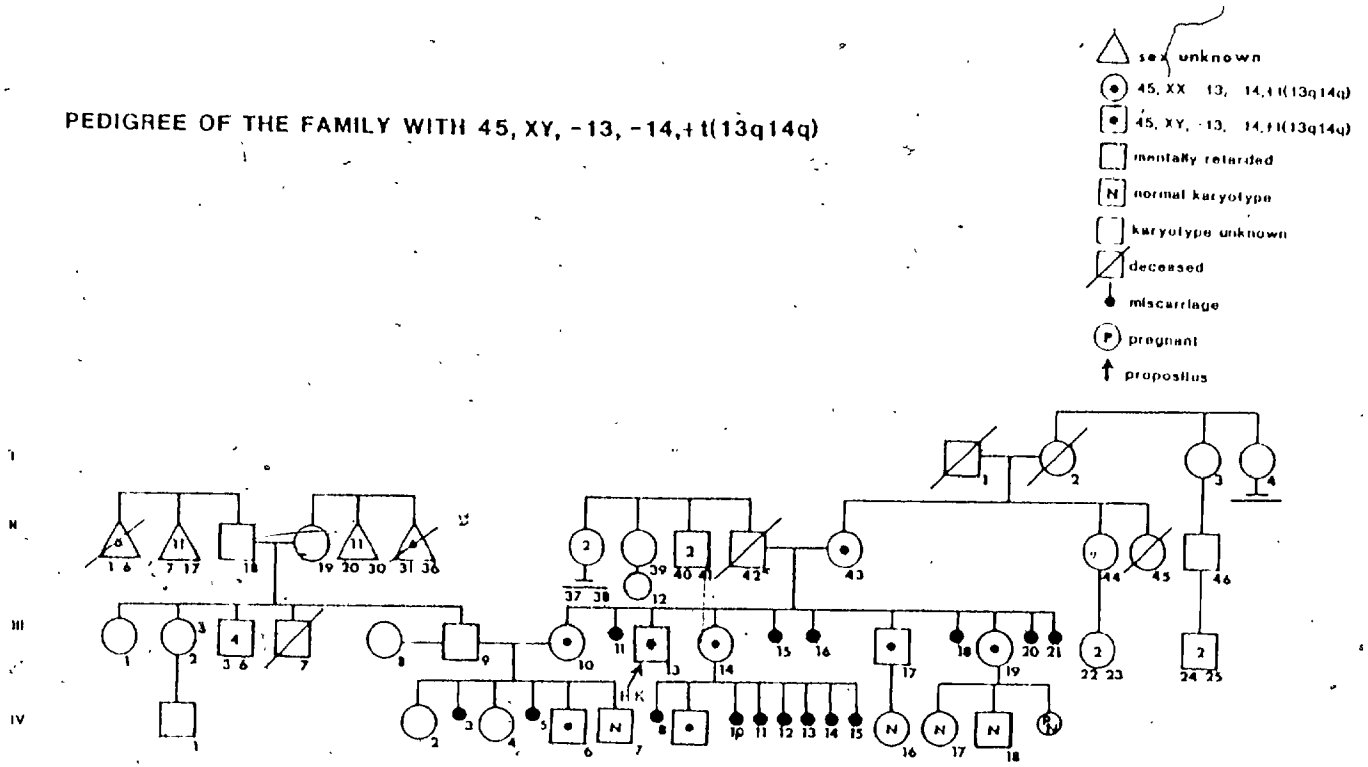

Figure 44. Karyotype of patient R.W., 46,XY, t(14;15)(q32;q13)

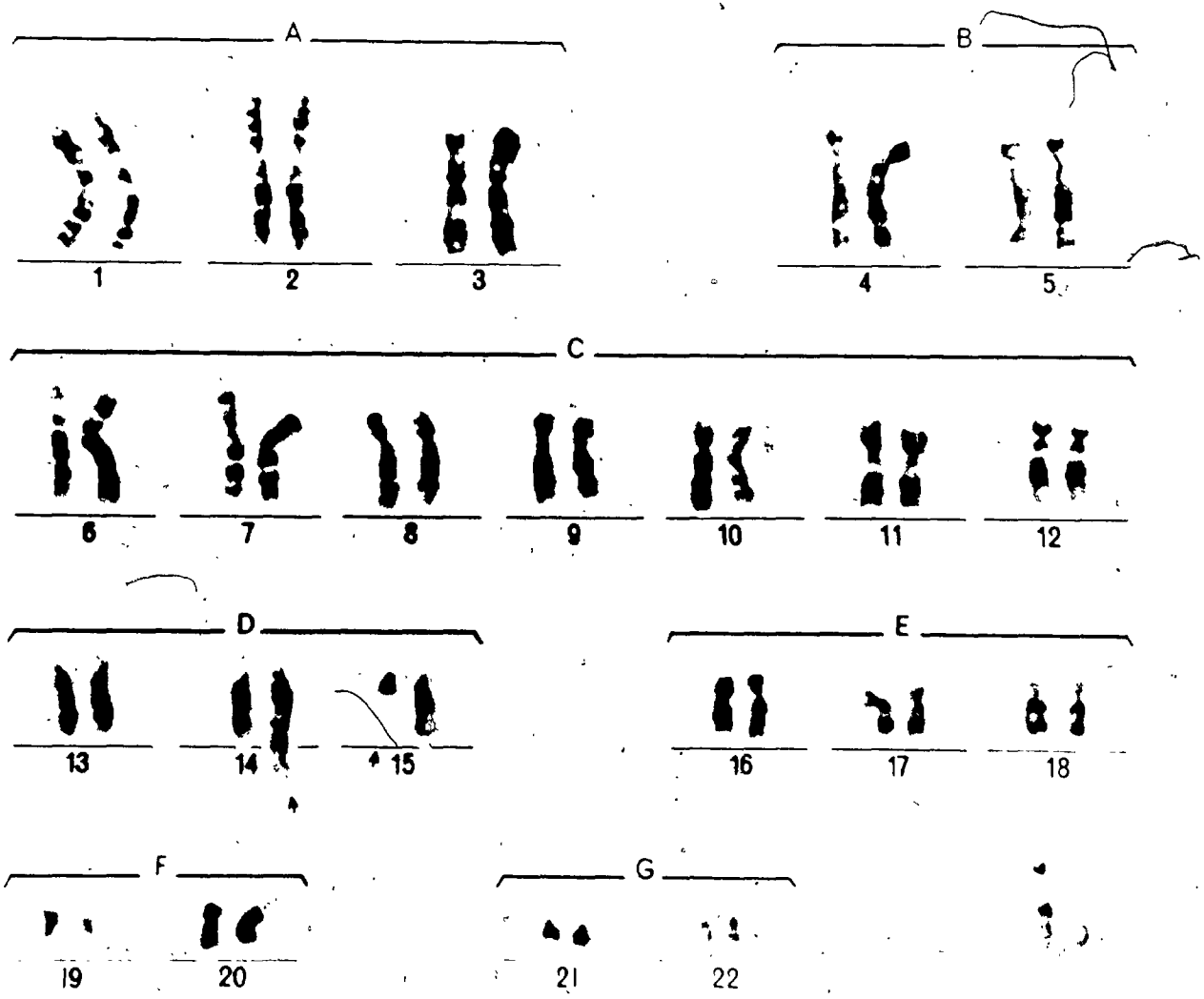
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Figure 45. Pedigree of the family W with a 46,XY, t(14;15)(q32;q13)

PEDIGREE OF THE FAMILY WITH A 46, XY, t(14q+;15q-)

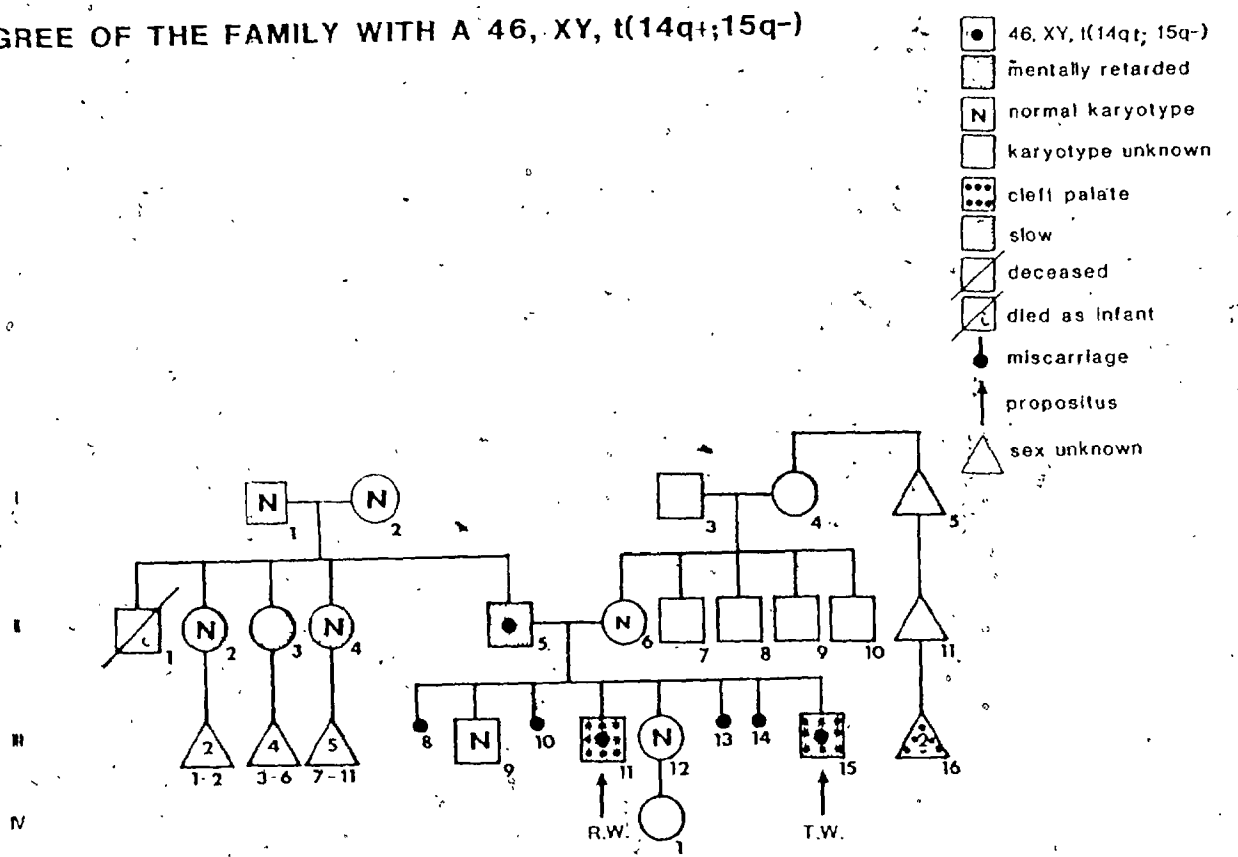

Figure 46. Meiotic configurations of the apparently balanced translocation, 46,XY,t(14;15)(q32;q13)

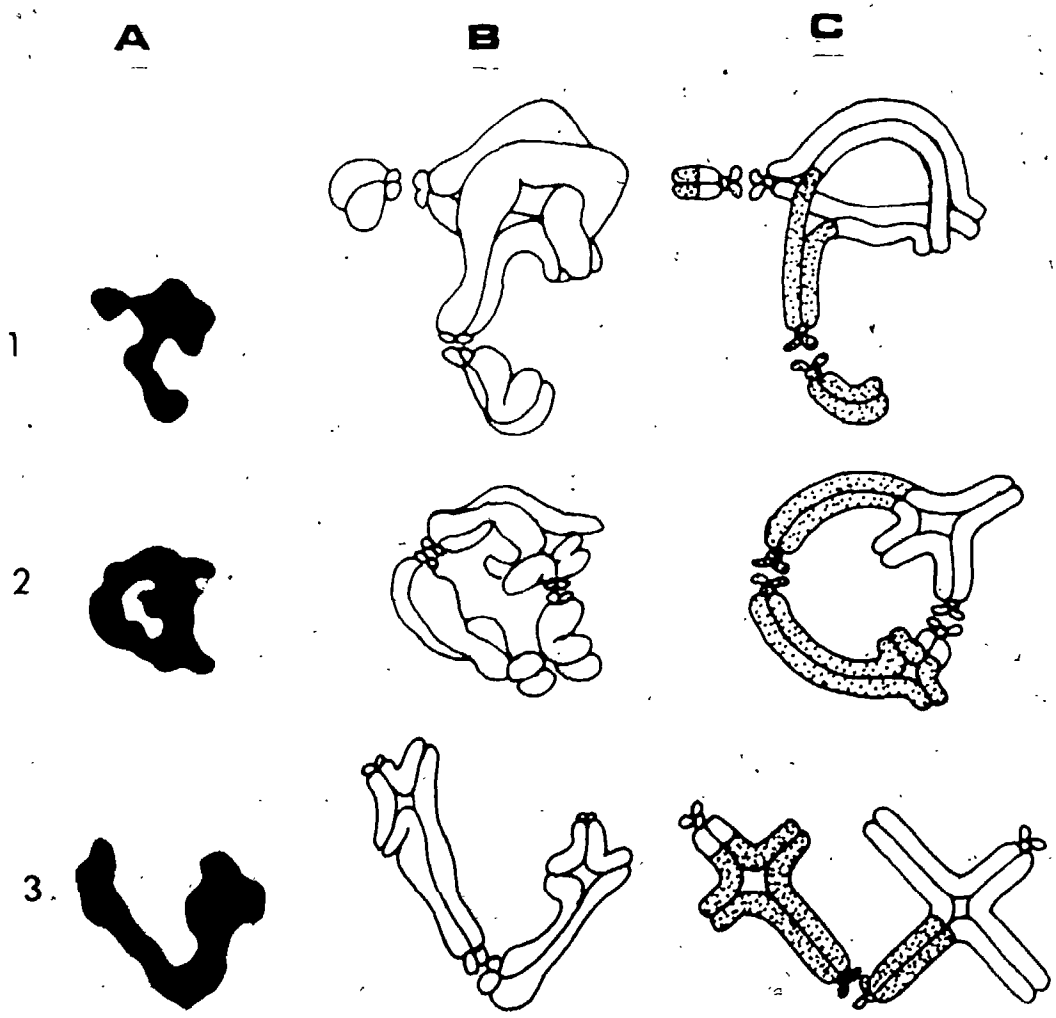
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Figure 47. Karyotype of patient B.V., 46,XY,t(5;14)(q22;q31)

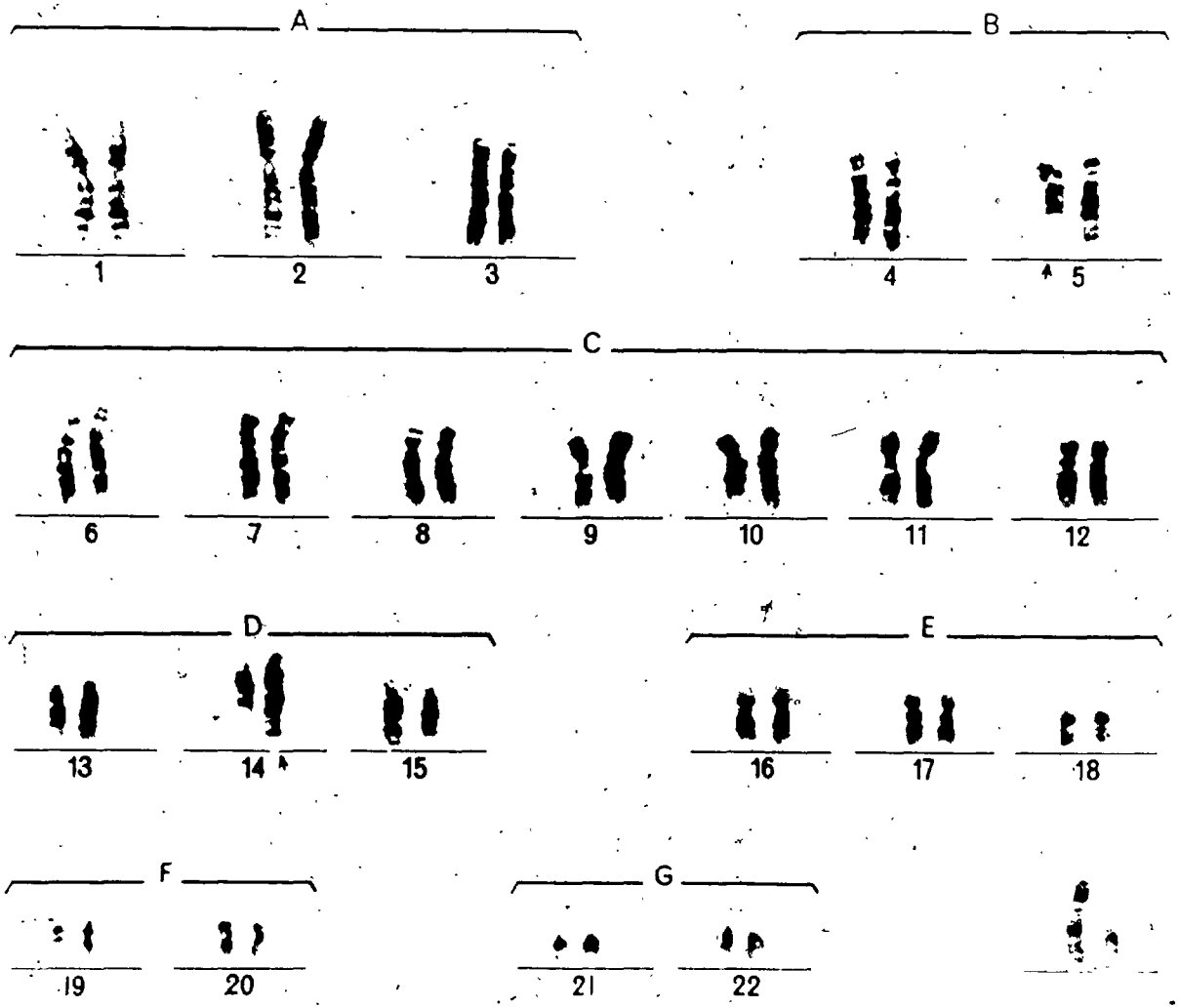
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Figure 48. Pedigree of the family V with a 46,XY, t(5;14)(q22;q31)

PEDIGREE OF THE FAMILY WITH A 46, XY, t(5q-; 14q+)

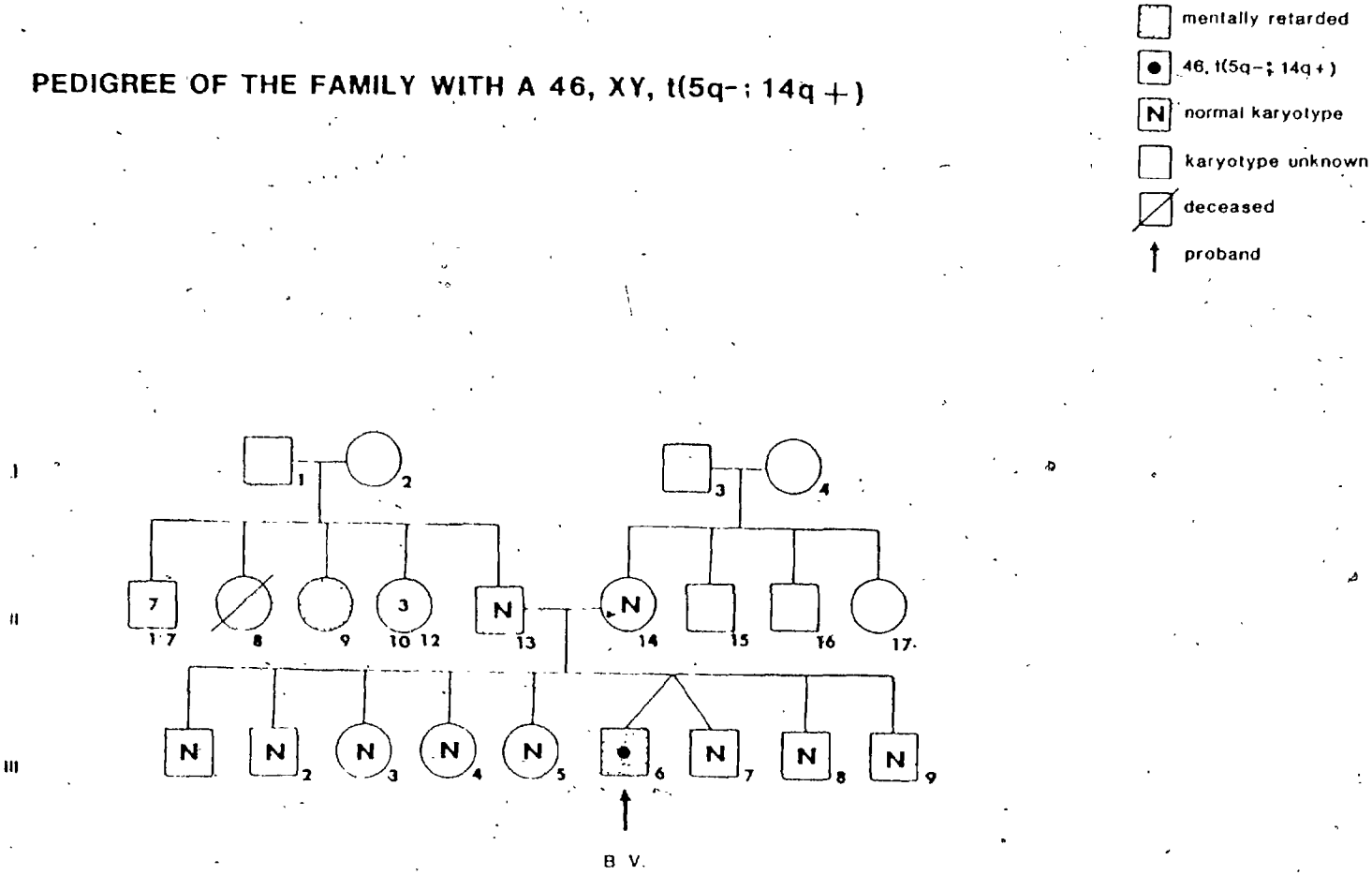

Figure 49. Karyotype of patient K.S., 46,XY,t(16;22)(p12;q13)

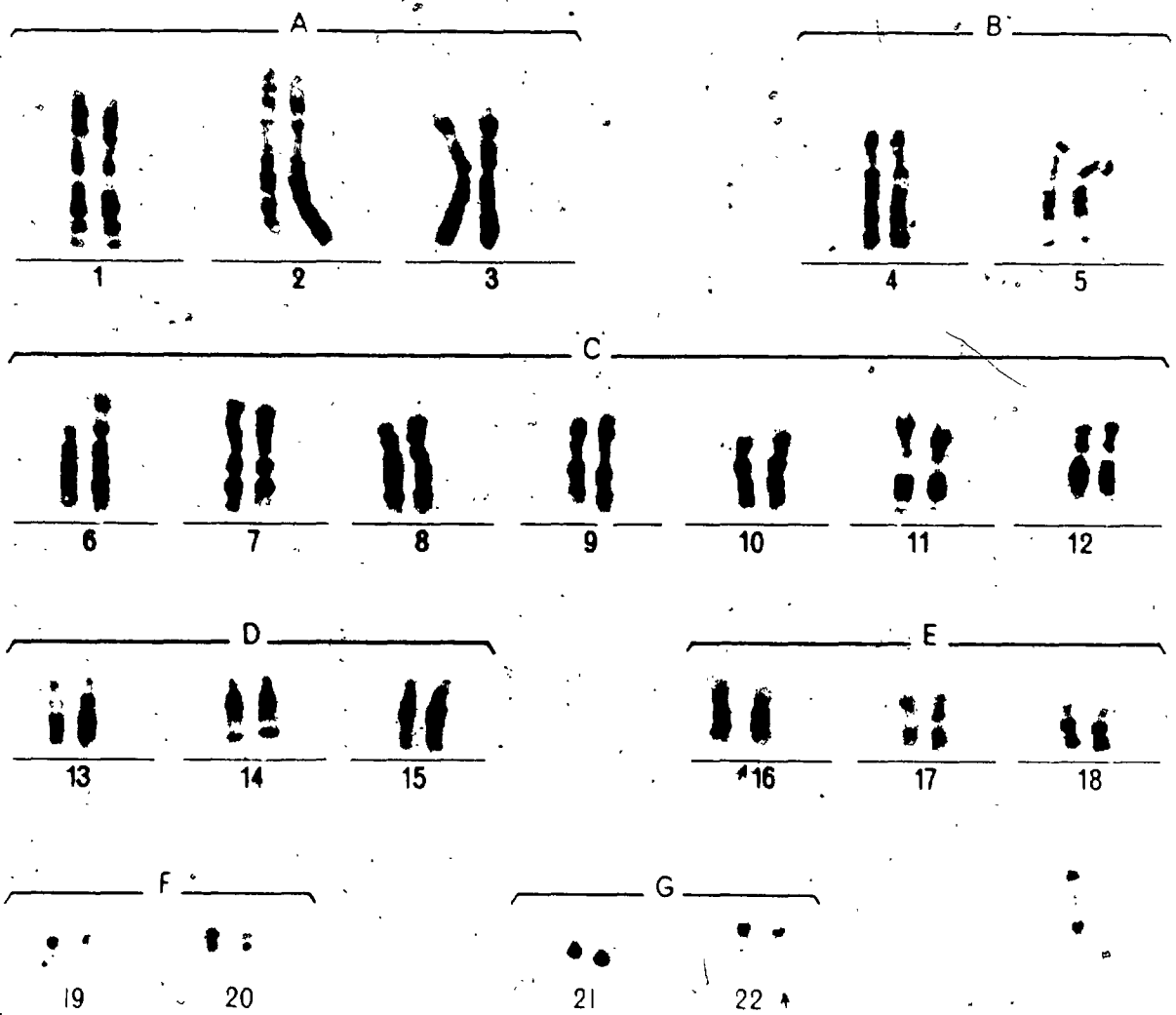
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Figure 50. Pedigree of a family S with a 46,XY,t(16;22)(p12;q13)

PEDIGREE OF A FAMILY WITH A 46, XY, t(16p-;22q+)

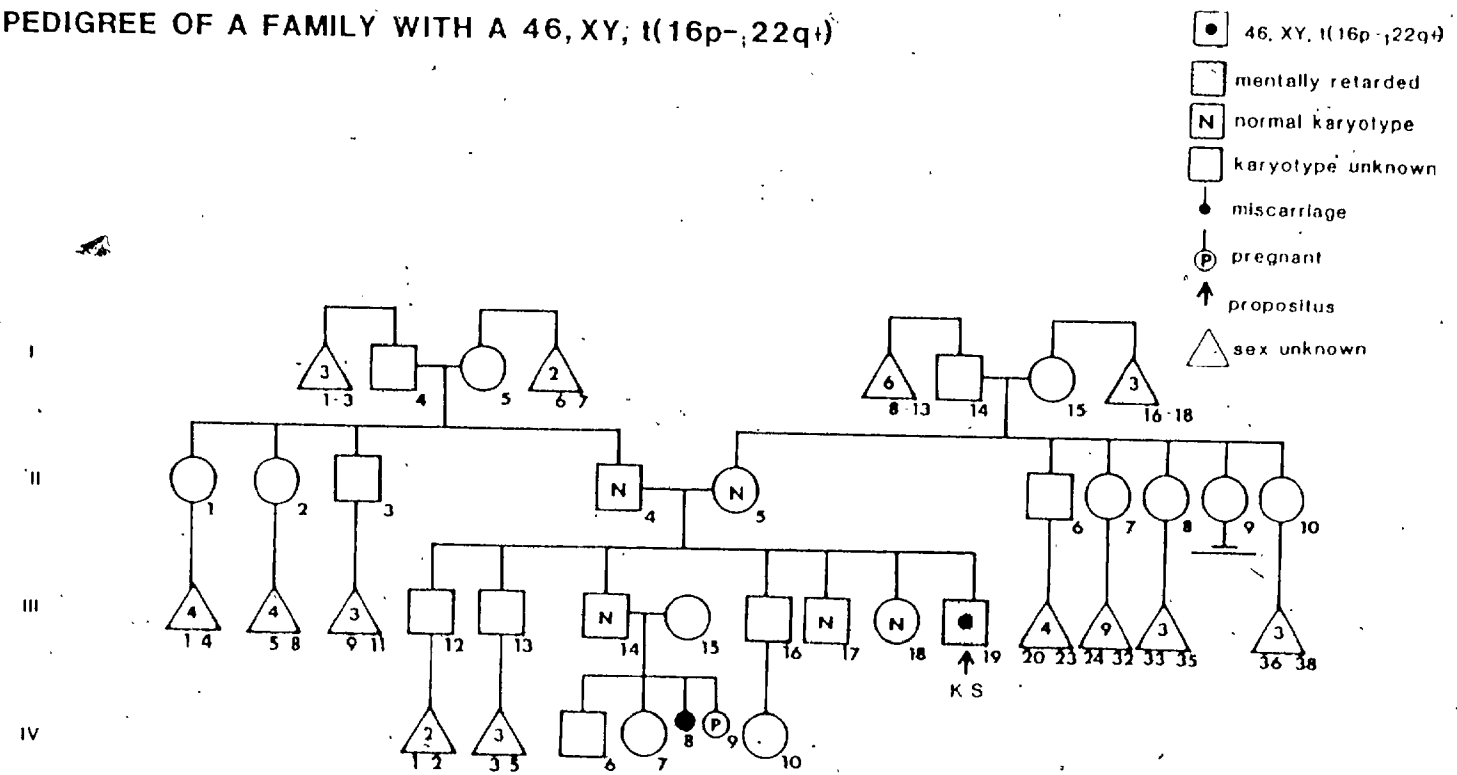

Figure 31. Karyotype of patient J.T., 46,XY,t(7;16)(q11;p13)


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Figure 52. Karyotype of patient T.W., 46,XY,der(8q+)?

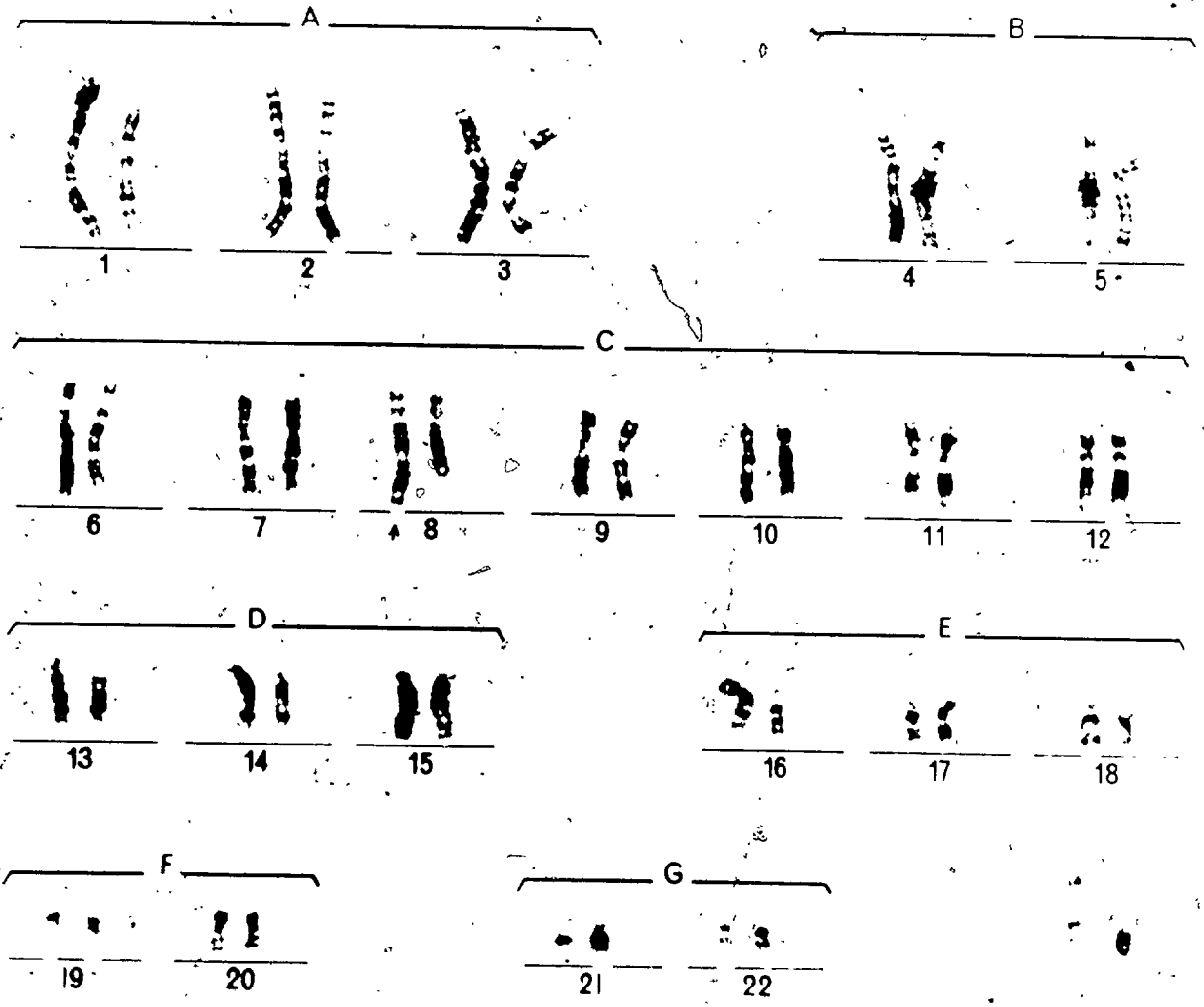
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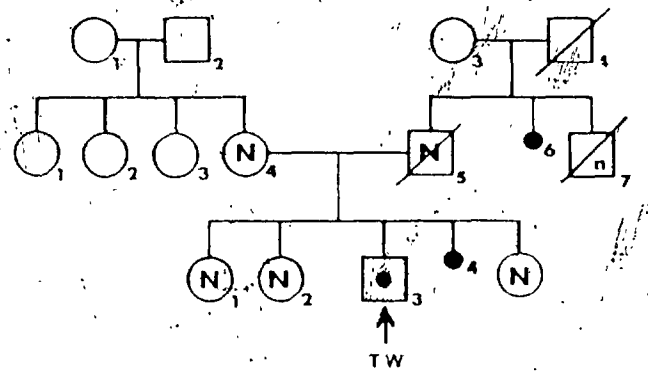
Figure 13. Pedigree of a family M with a 46,XY,der(8q+)?

PEDIGREE OF A FAMILY WITH A 46,XY, 8q+



- 46, XY, 8q+
- mentally retarded
- N normal karyotype
- karyotype unknown
- n died as newborn
- deceased
- miscarriage
- propositus

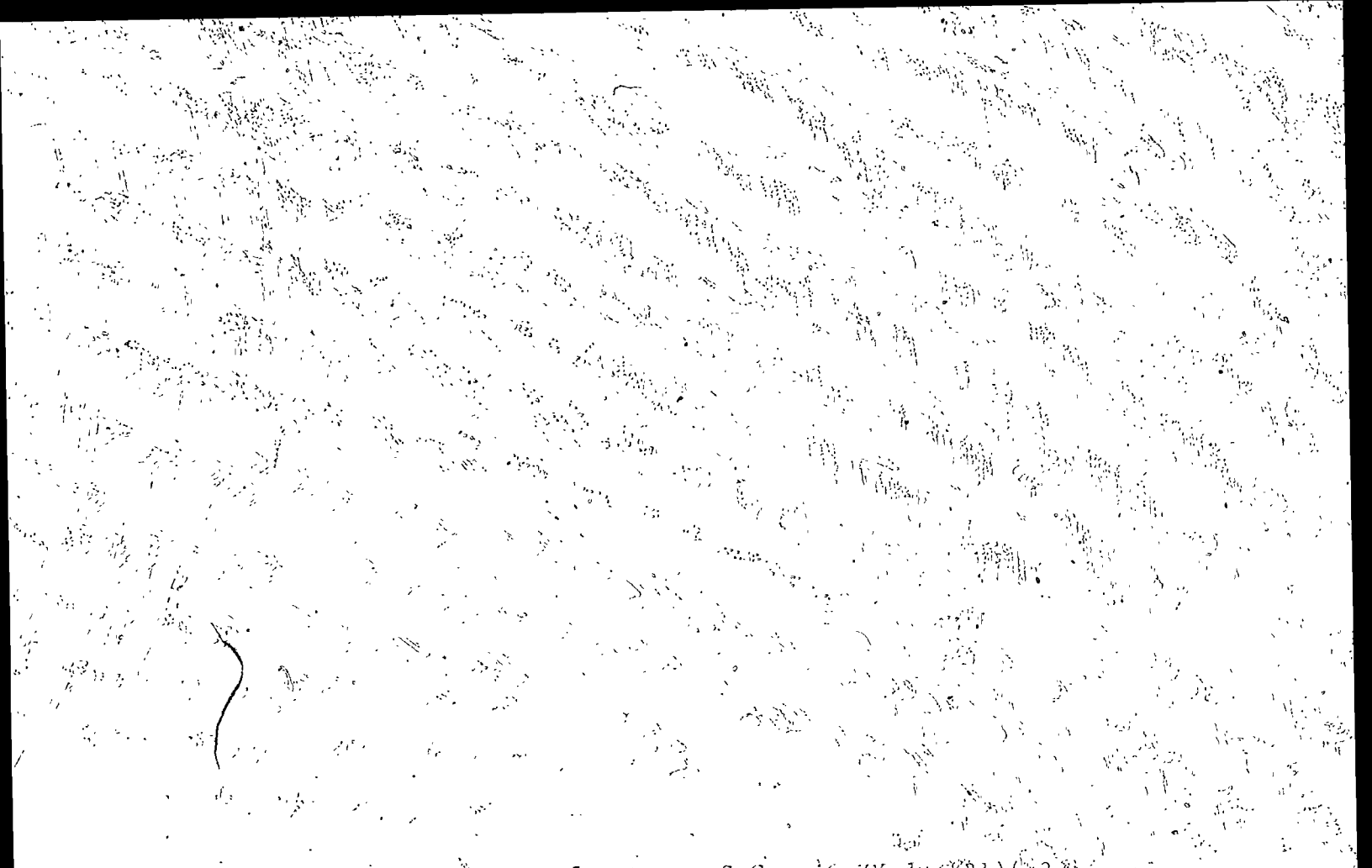


Figure 54. Karyotype of patient S.C., 46,XY,dup(21)(q22)

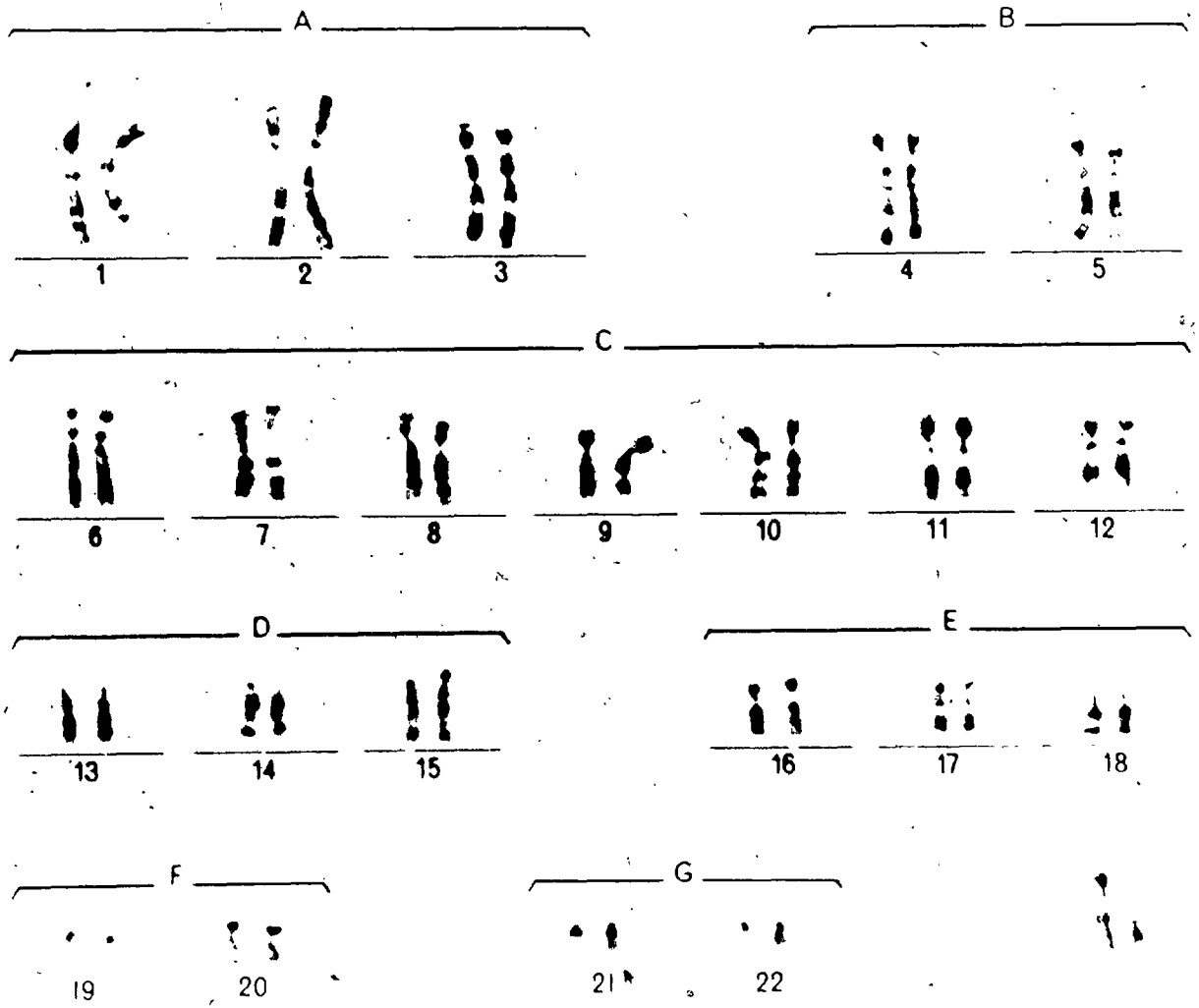

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Figure 55. R-banding of the dup(21)(q22)

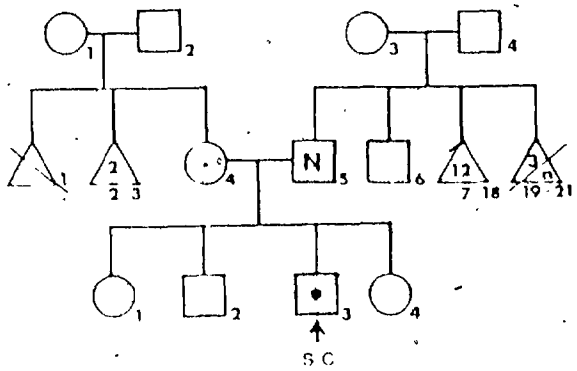
R-BANDING

S.C.

21 21dup(q22)

Figure 56. Pedigree of a family Cn with a 46,XY,dup(21)(q22)

PEDIGREE OF A FAMILY WITH A 46,XY, dup (21)(q22).



- 46 XX/47,XXX/48,XXXX
- 46, XY, dup (21)(q22)
- mentally retarded
- N normal karyotype
- karyotype unknown
- deceased
- n died as newborn
- propositus
- sex unknown

Figure 57. Karyotype of patient K.C., 46,XY,del(10)(p13→pter)

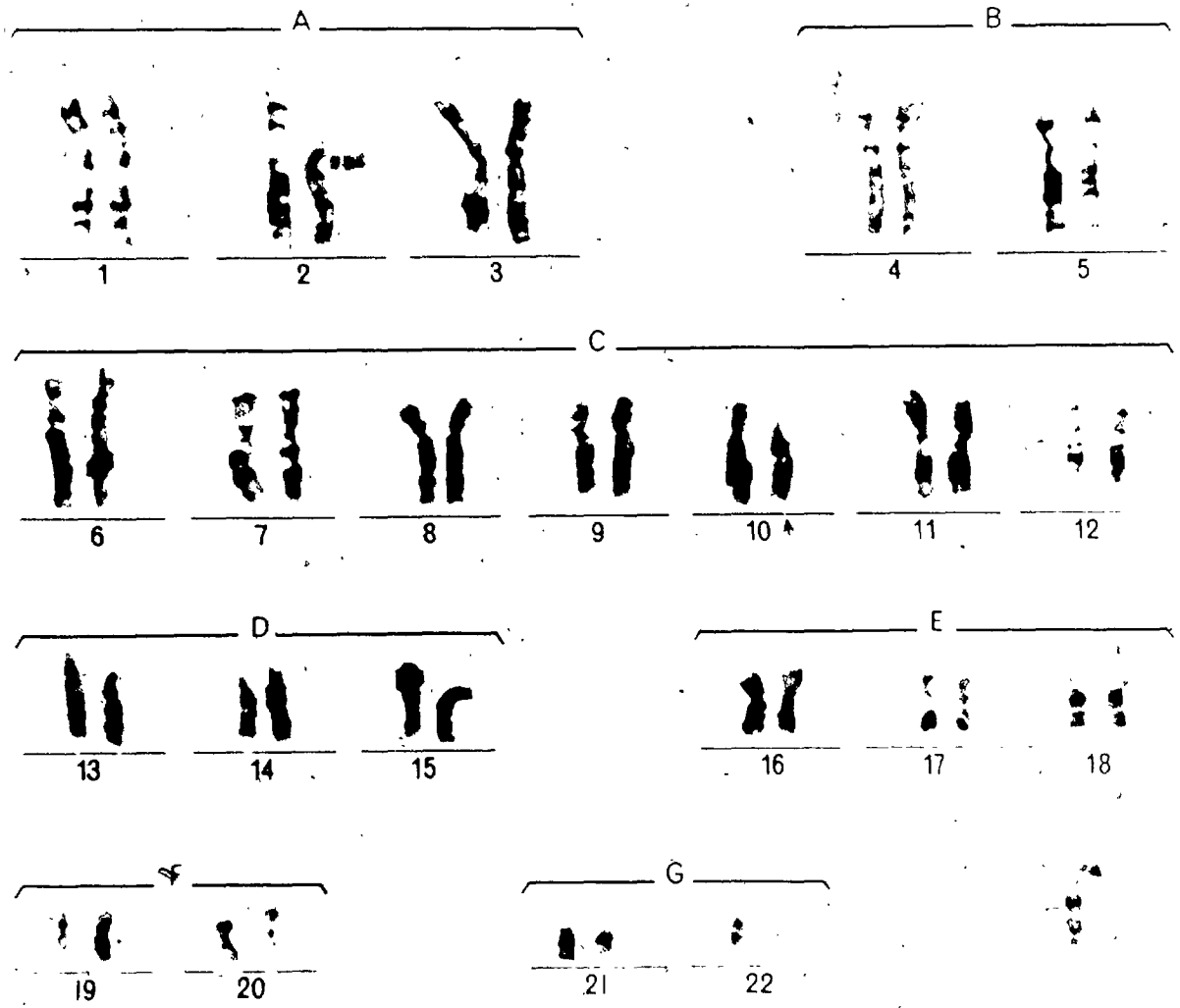

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Figure 58. Pedigree of a family C with a 46,XY,del(10)(p13-pter)

PEDIGREE OF A FAMILY WITH A 46, XY, 10p-

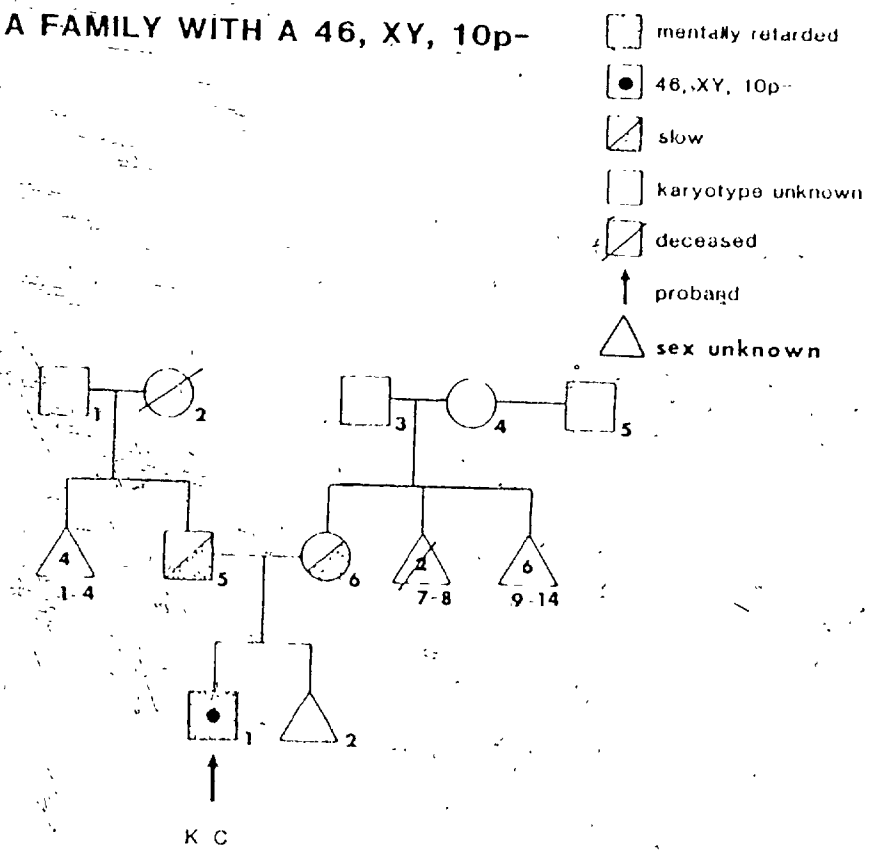

Figure 59. Karyotype of patient R.Mc., 47,XY,+inv dup(15)(p13→q12)

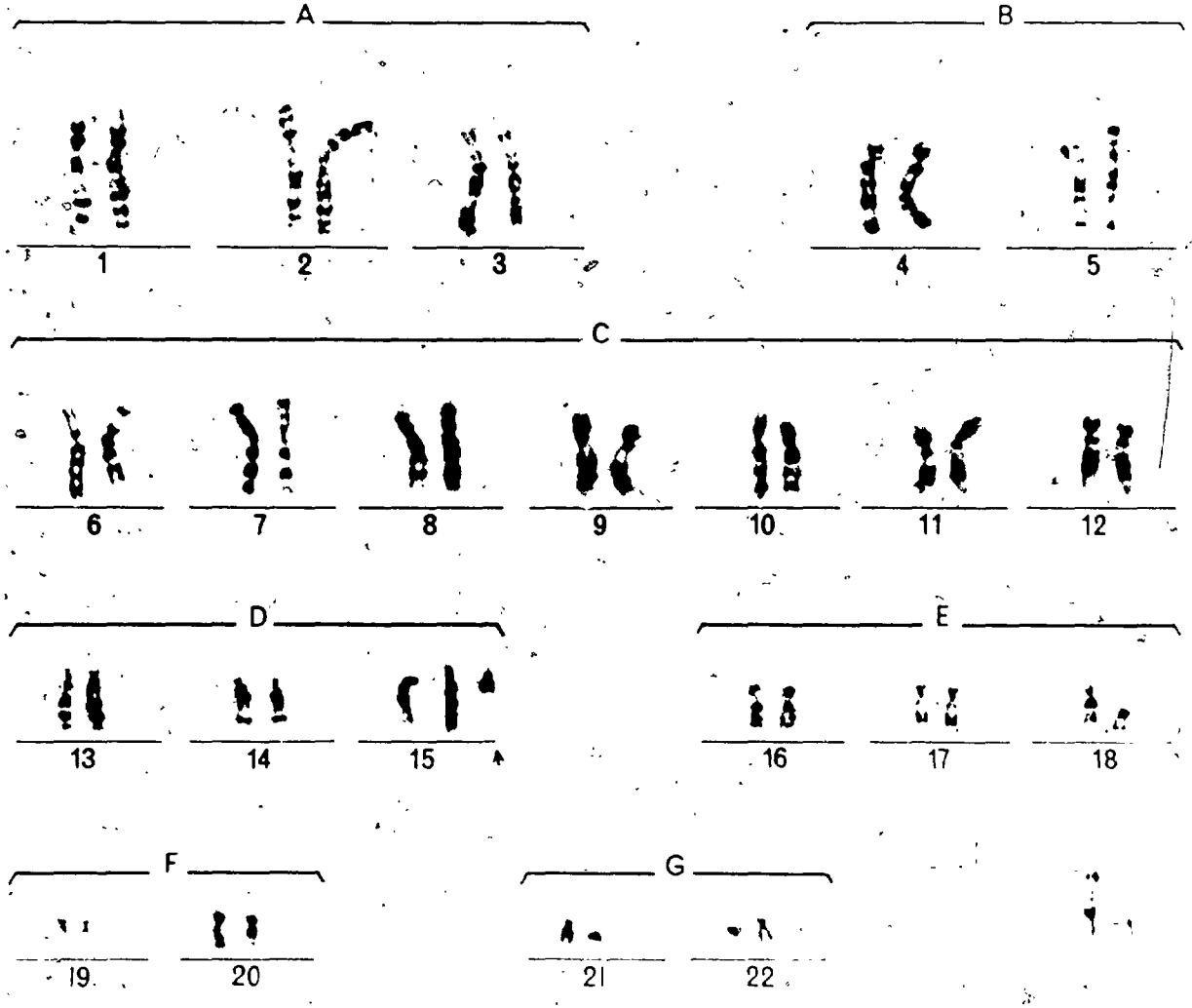
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Figure 60. Karyotype of patient H.W., 47,XY,+inv dup(15)(p13→q12)

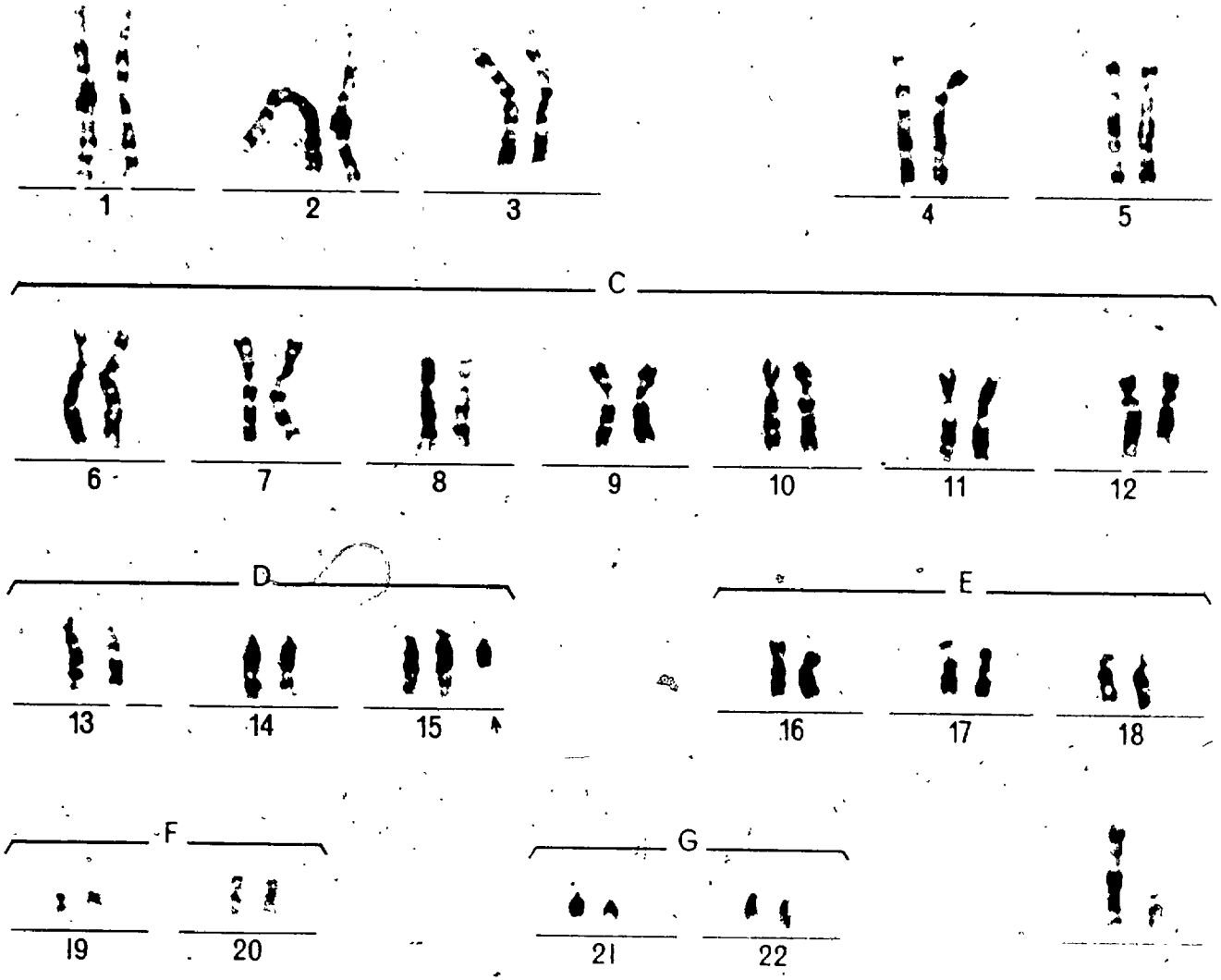
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Figure 61. Karyotype of patient C.V., 47,XY,+inv dup(15)(p13-q12)

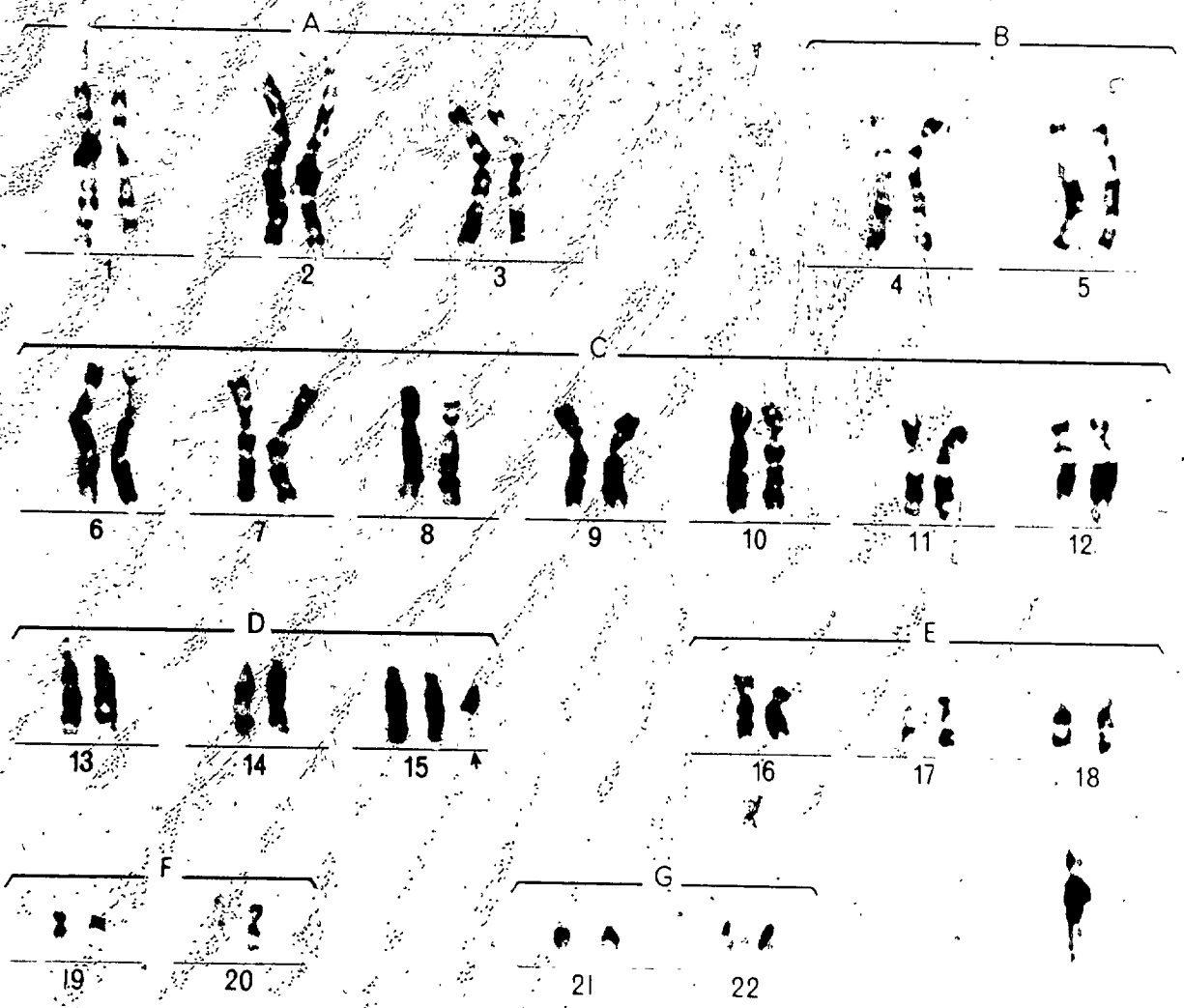
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Figure 62. C- and NOR-banding of the inv dup(15) (p13 → q12)

C-BANDING

C.V. H.W. R.M.

NOR-BANDING

C.V. H.W. R.M.

Figure 63. Methyl green/DAPI C-bands

A. Specific C-banding involving only chromosome 15 of the D and G groups.

B. C-banding involving both centromeres of inv dup (15).

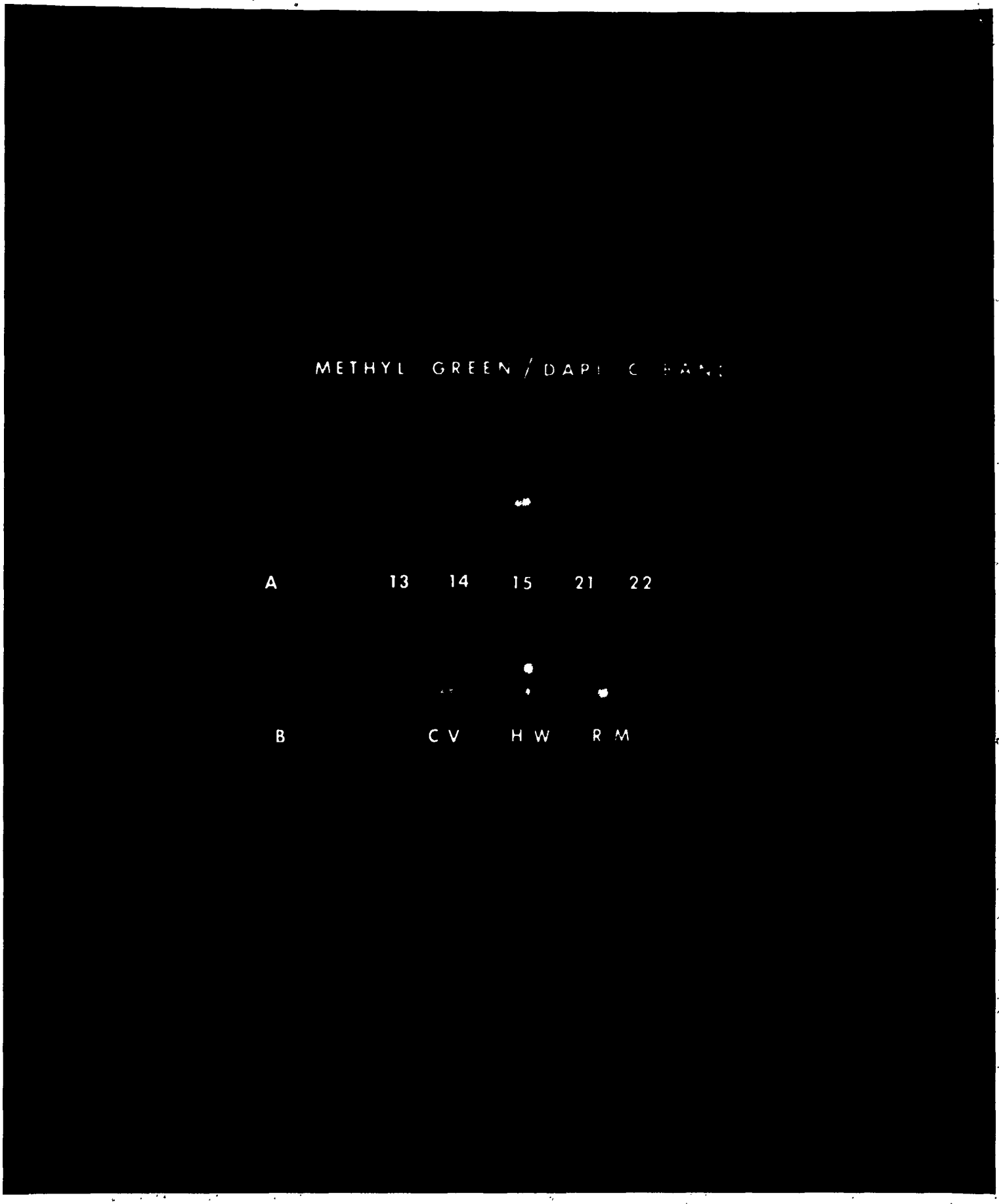
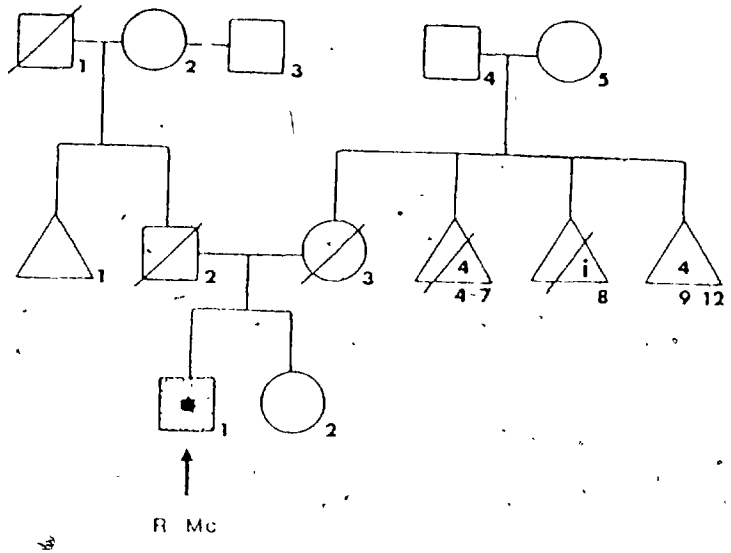

METHYL GREEN / DAPI
A
13
14
15
21
22
B
CV
HW
RM

Figure 64. Pedigree of family Mc with a 47,XY, +inv dup(15)(p13→q12)

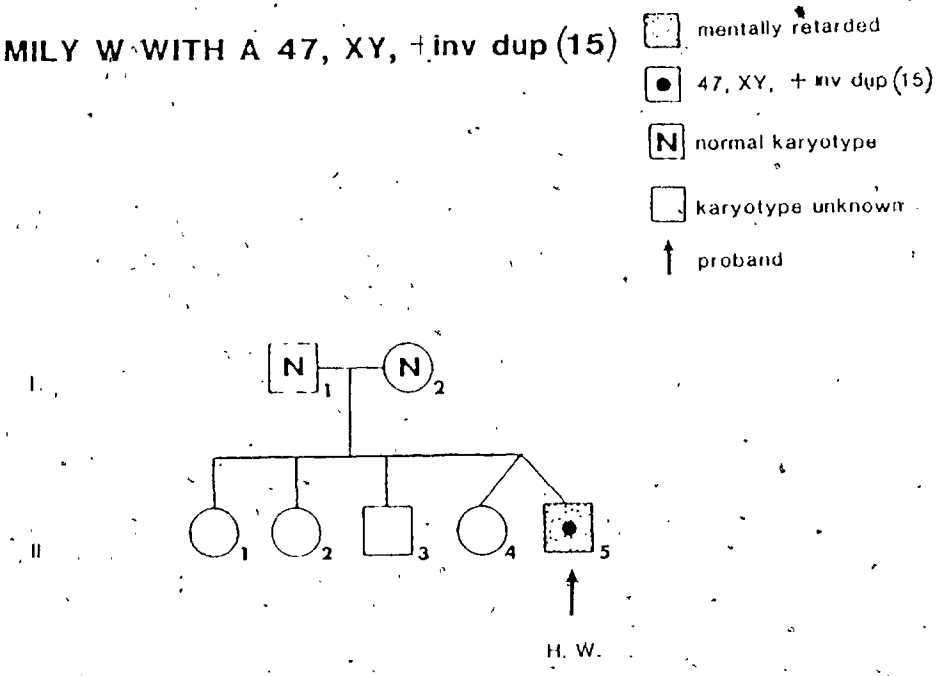
PEDIGREE OF FAMILY Mc. WITH A 47, XY, +inv dup(15)



mentally retarded
47,XY, +inv dup(15)
karyotype unknown
deceased
died as infant
proband
sex unknown

Figure 65. Pedigree of family W with a 47,XY, +inv dup(15)(p13→q12)

PEDIGREE OF FAMILY W WITH A 47, XY, + inv dup (15)



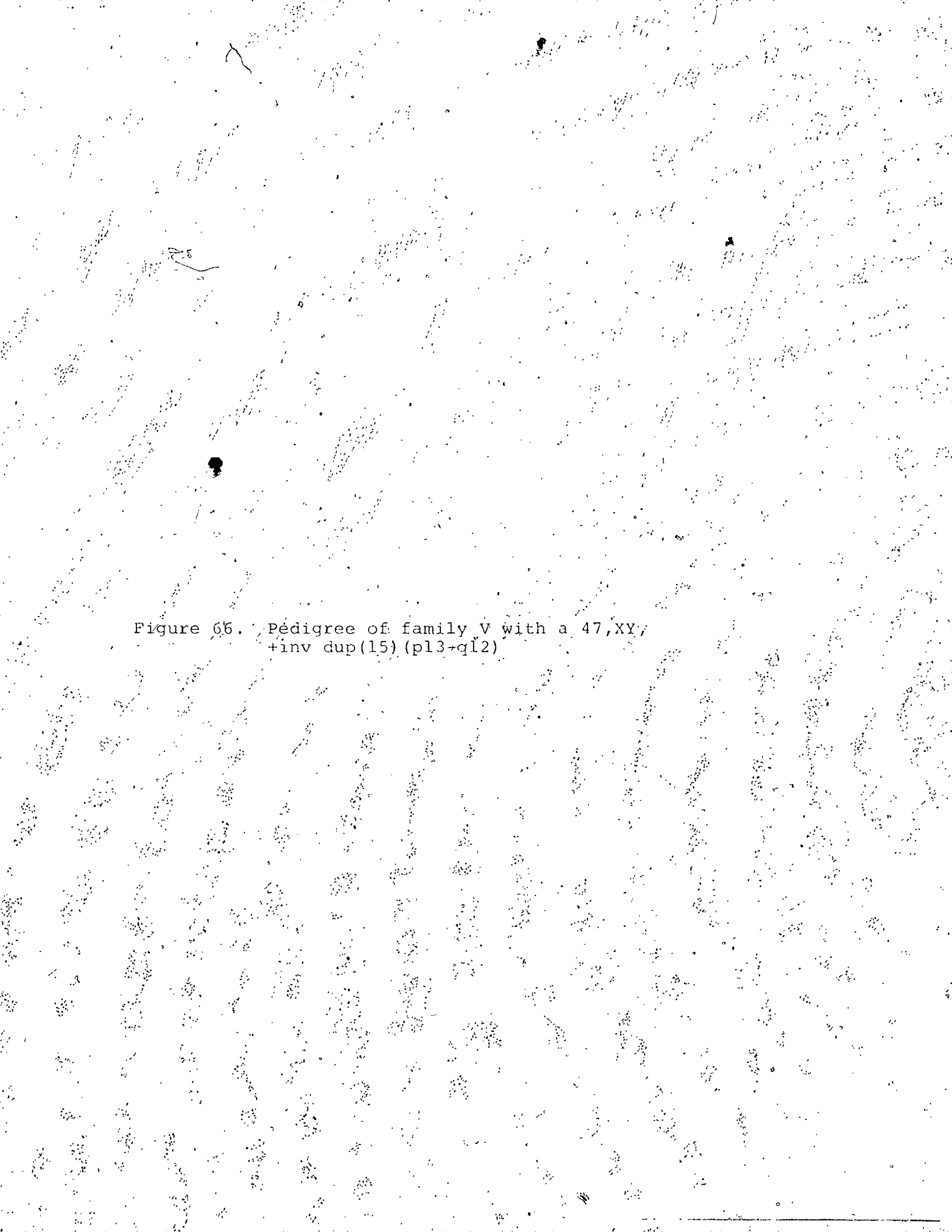
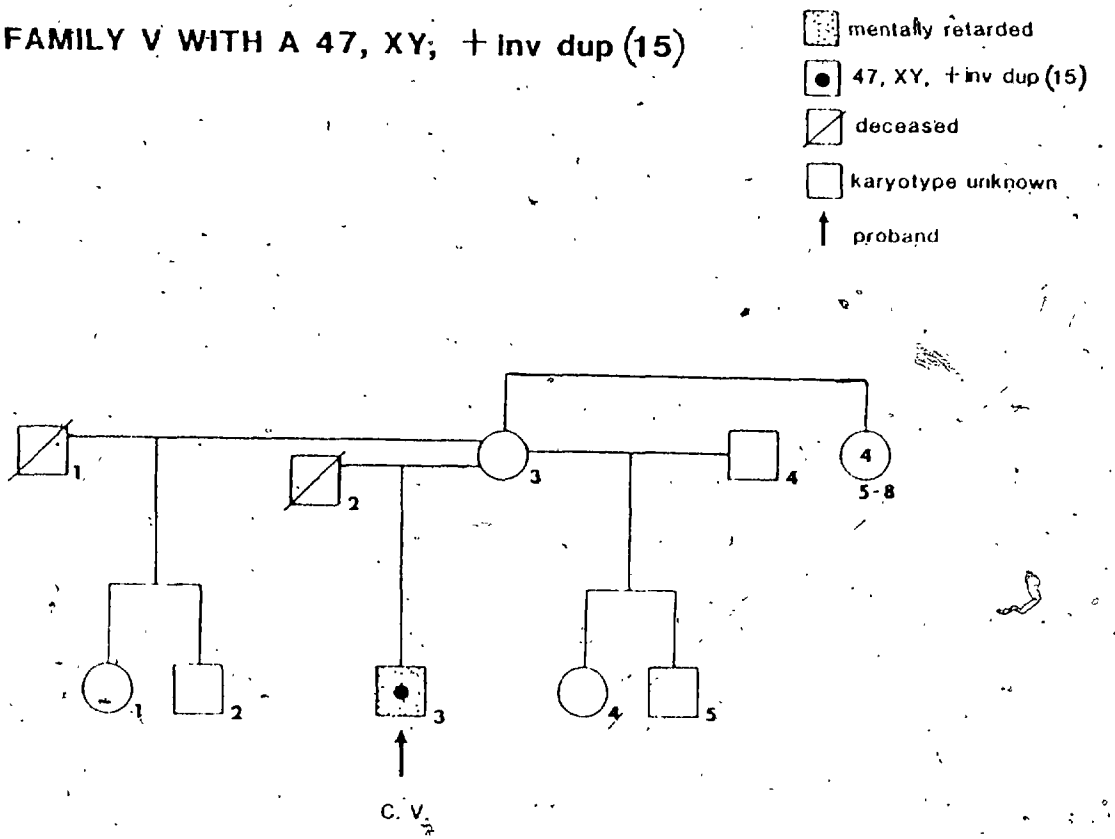


Figure 66. Pedigree of family V with a 47,XY, +inv dup(15)(p13→q12)

PEDIGREE OF FAMILY V WITH A 47, XY; + inv dup (15)



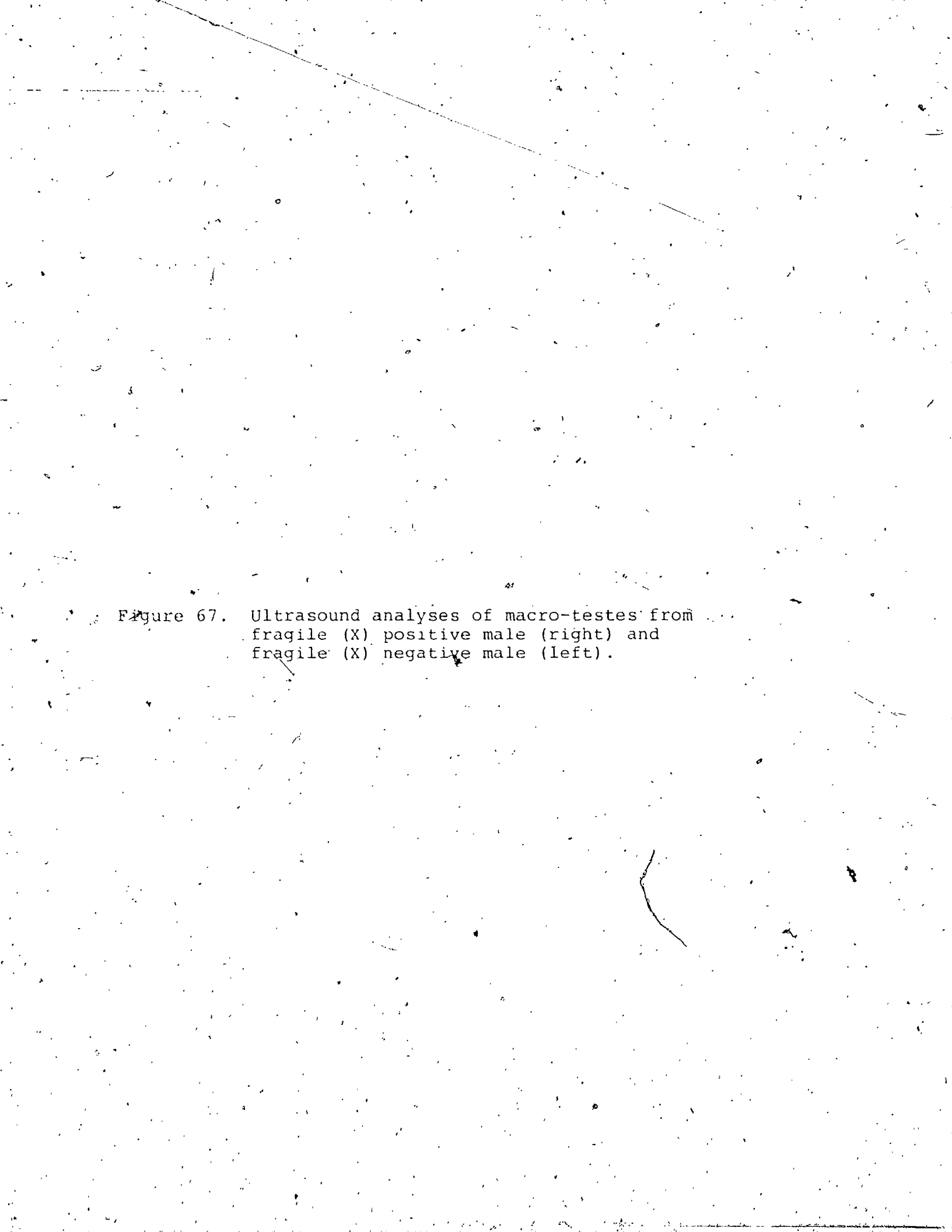


Figure 67. Ultrasound analyses of macro-testes from fragile (X) positive male (right) and fragile (X) negative male (left).


LNF
TESTE

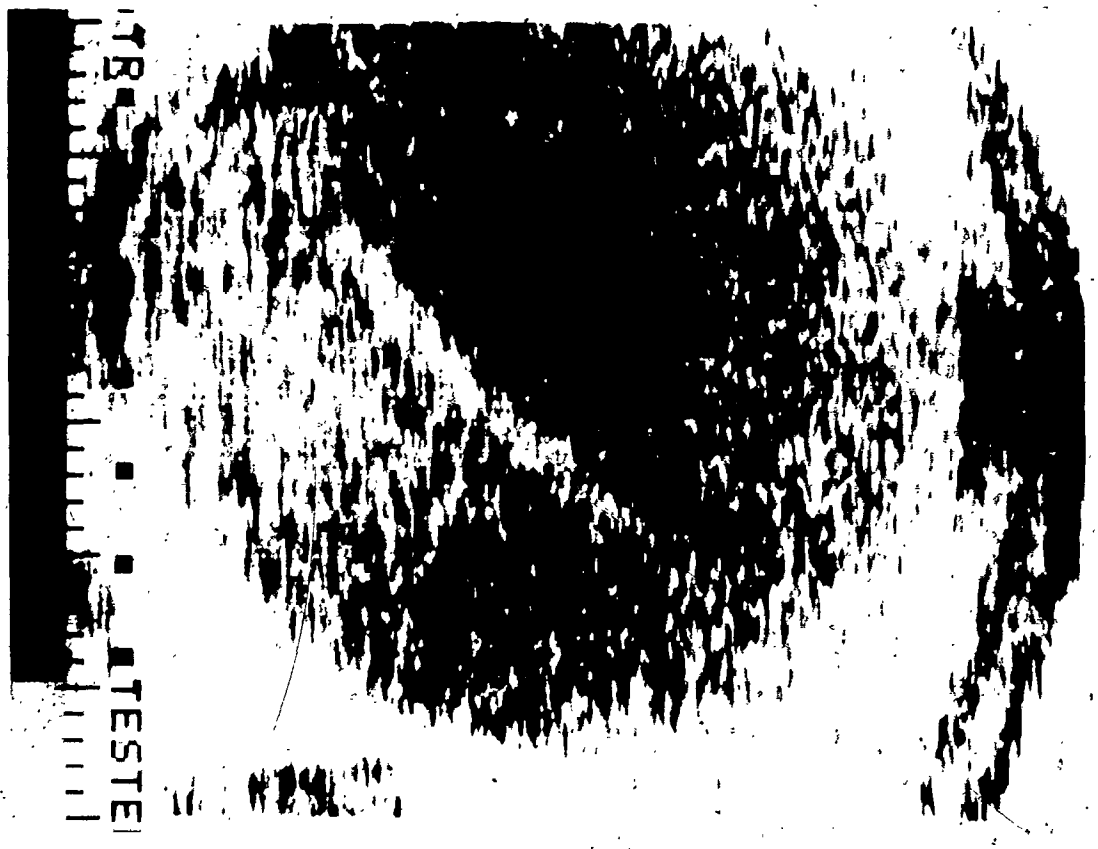

TESTE

Table 1. Comparison Between The Surveys of Speed et al (1976) and Rasmussen et al (1982).

	Speed et al (1976)	Rasmussen et al (1982)
Total number karyotyped	2,770	1,905
Frequency of chromosome abnormalities in Relation to total karyotyped	$\frac{297}{2,770}$ = 10.7%	$\frac{359}{1,905}$ = 18.8%
Sex chromosome	31 (1.4%)	33 (1.8%)
Down syndrome	250 (9.0%)	281 (14.7%)
Other trisomies (full or partial)	9 (0.3%)	16 (0.8%)
Deletions or rings	7 (0.3%)	11 (0.6%)
Balanced re-arrangements	0	18 (0.9%)
Total Population of Survey Area	480,000	560,000
Number of mentally retarded individuals	3,020	2,157
Prevalence of MR	0.63%	0.39%
Frequency of chromosome abnormalities in relation to total population	$\frac{297}{480,000}$ = 0.067%	$\frac{359}{560,000}$ = 0.064%
Sex chromosome	0.0065%	0.0059%
Down syndrome	0.052%	0.058%
Other trisomies (full or partial)	0.0019%	0.0029%
Deletions or rings	0.0015%	0.0020%
Balanced rearrangements	0	0.0032%

Table 2. Surveys of Institutions for the Mentally Retarded (Unselected Populations)@

Reference	Population	Total # Examined	Sex Chromosome Abnormalities #	%	Down's Syndrome #	%	Autosomal aneuploidies & unbalanced Re-arrangements #	%	Deletions & Rings #	%	Balanced Rearrangements #	%	Fra(X) #	%	TOTAL #	%
Sutherland and Wiener (1971)	Institutionalized MR children	159	0	0	17	10.7	3	1.9	0	0	0	0	-	-	20	12.6
Newton et al (1972a,b)	Institutionalized patients ≥ 15 yrs.	1,255	10	0.8	103	8.2	7	0.6	3	0.2	5	0.4	-	-	128	10.1
Fujita and Fujita (1974)	Institutionalized MR children	59	2	3.4	1	1.7	1	1.7	0	0	0	0	-	-	4	6.8
Cassinen et al (1975)	Institutionalized children, IQ < 50	867	3	0.3	111	12.9	7	0.8	4	0.5	4	0.5	-	-	129	15.0
Sutherland et al (1976)	All patients in Institution	588	4	0.7	73	12.6	5	0.9	4	0.7	4	0.7	-	-	90	15.3
Jacobs et al (1978)*	All patients in Institution ≤ 27 yrs.	475	3	0.6	40	8.4	5	1.0	4	0.8	5	1.0	-	-	57	12.0
Ally and Grace (1979)*	All patients in Institution	512	3	0.6	42	8.2	3	0.6	4	0.8	4	0.8	-	-	57	11.1
Faed et al (1979)+	All patients in Institution	756	6	0.8	91	12.0	2	0.3	2	0.3	2	0.3	-	-	103	13.6
Gripenberg et al (1980)+	All patients in Institution	1,082	10	0.9	305	28.7	17	1.6	14	1.3	4	0.4	-	-	350	32.9
Kondo et al (1980)+	All patients in Institution	449	1	0.2	33	7.4	1	0.2	1	0.2	1	0.2	-	-	37	8.2
Klein et al (1980)*	All patients in Institution	182	3	0.6	25	14.3	0	0.0	0	0.00	0	0.0	-	-	28	14.9
Brondum-Nielsen et al (1983c)*	All patients in Institution	476	4	1.1	58	12.2	6	1.1	2	0.4	11	0.6	-	-	81	17.0
Venter and Op't Hof (1982)*	Patients in various Institutions	2,533	unspecified		397	15.7	←		unspecified		→		22	0.9	525	20.6
TOTAL		6,850	49	0.7	899	13.2	57	0.8	38	0.6	40	0.6			1,084	15.9
		9,353 (including Venter and Op't Hof (1982))			1,296	13.8									1,609	17.2

* banded survey
+ partially banded survey
@ based on Jacobs et al (1978)

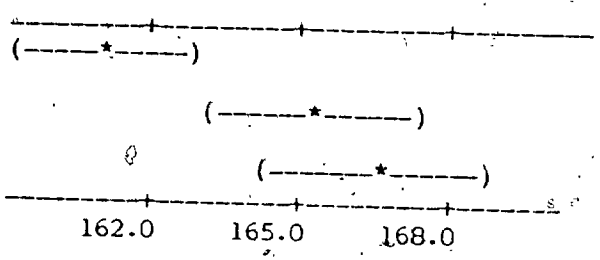
Table 3. Selected Surveys of Institutions for the Mentally Retarded (Selected Populations)@

Reference	Population	Total # Examined	Sex Chromosome Abnormalities #	Sex Chromosome Abnormalities %	Autosomal aneuploidies & Unbalanced Re-arrangements #	Autosomal aneuploidies & Unbalanced Re-arrangements %	Deletions and Rings #	Deletions and Rings %	Balanced Re-arrangements #	Balanced Re-arrangements %	TOTAL #	TOTAL %
Madison Blind Study (Summitt, 1969; Daly, 1970; Doyle, 1976; Magnelli, 1976)	Institutionalized children, IQ < 75, 3 or more congenital abnormalities with no recognized syndrome	240	2	0.8	11	4.6	7	2.9	2	0.8	22	9.2
Chan et al (1970)	MR with low birth weight	134	6	4.4	4	3.0	1	0.7	1	0.7	12	9.0
Carey et al (1971)	MR with multiple congenital anomalies; MR with familial MR/miscarriage; MR and rare dans	223	5	2.2	5	2.2	4	1.8	1	0.4	15	6.7
Carrel et al (1973)	Moderate to profound MR, multiple congenital abnormalities with no known syndrome	121	1	0.8	12	9.9	5	4.1	1	0.8	19	15.7
Lubs and Lubs (1973)*	MR children with no recognized syndrome	54	0	0	4	7.4	0	0	0	0	4	7.4
Erdtmann et al (1975)	Institutionalized children, IQ < 75, 3 or more congenital abnormalities with no recognized syndrome	51	1	2.0	2	3.9	2	3.9	0	0	5	9.8
Therapel & Summitt (1977)*	MR with multiple congenital anomalies and no known syndrome	200	0	0	3	1.5	0	0	3	1.5	6	3.0
	(Normal Controls)	200	0	0	0	0	0	0	0	0	0	0
Moghe et al (1981)*	MR with at least 2 minor and 1 major physical malformation with no known syndrome	74	7	9.5	1	1.4	5	6.8	1	1.4	14	18.9
Nelson and Smart (1982)*	MR with physical characteristics associated with chromosome anomalies	595	7	1.2	6	1.0	6	1.0	2	0.3	21	3.5
Kodama (1982)*	MR with physical handicaps	193	0	0	1	0.5	0	0	3	1.6	4	2.1
Coco and Penchaszadeh (1982)*	Institutionalized children, 3 or more congenital anomalies with no known syndrome	200	5	2.5	23	11.5	12	6.0	2	1.0	42	21
Retief et al (1983)*	MR with non-specific congenital abnormalities	1,022			Unspecified						109	
Total		2,163	34	1.6	72	3.3	42	1.9	16	0.74	154	7.1
		3,185 (Including Retief et al, 1983)									263	8.3%
												7.7%

* banded surveys
@ based on Jacobs et al (1978)

Table 4. 95% Confidence Intervals for Group Mean Heights

GROUP	MEAN HEIGHT (cm)	STANDARD DEVIATION	95% CONFIDENCE INTERVAL USING POOLED STANDARD DEVIATION
Micro-orchid	161.2**	± 13.1	± 1.8
Normal	165.2	± 9.3	± 2.1
Macro-orchid	167.1	± 11.7	± 2.3
TOTAL	164.1	± 11.9	



pooled standard deviation = 11.7 cm

*statistically significant difference (analysis of variance: $F = 9.39$, $\alpha = .05$; Tukey's test: $q = 164.7$, $\alpha = .05$)

**statistically significant from mean height of fra(X) positive males (169.3 ± 6.5 cm) (Table 13) (analysis of variance: $F = 8.06$, $\alpha = .05$; Tukey's test: $q = 165.4$, $\alpha = .05$)

Table 5. Correlation Co-efficients for Various Physical Features of Adult (non-Down Syndrome) MR Males

	Height	Macro-Orchidism	Height	Micro-Orchidism	Height	Normal Testicular Volume
Age	.122	-.129	-.015	.078	-.275**	.179
Height	-	-.042	-	.177*	-	.145

* $p < .05$
** $p < .01$

Table 6. The Distribution of Testicular Volume According to Diagnostic Categories

Category	Macro-orchidism	Micro-orchidism	Normal
1. Chromosome Abnormalities [Non-fra(X)]	4 (2.4%)	7 (4.2%)	5 (3.0%)
2. Non-specific XLMR			
Fra(X) positive	17 (10.3%)	2 (1.2%)	2 (1.2%)
Fra(X) negative	18 (10.9%)	16 (9.7%)	15 (9.1%)
3. Other Genetic Factors			
Consanguinity	3 (1.8%)	2 (1.2%)	4 (2.4%)
Autosomal dominant	2 (1.2%)	1 (0.6%)	4 (2.4%)
Autosomal recessive	4 (2.4%)	3 (1.8%)	2 (1.2%)
Syndromes of unknown etiology	0	1 (0.6%)	1 (0.6%)
Non-specific familial MR	16 (9.7%)	18 (10.9%)	16 (9.7%)
4. Environmentally Caused			
Pre-natal disease	2 (1.2%)	5 (3.0%)	2 (1.2%)
Post-natal disease	4 (2.4%)	18 (10.9%)	11 (6.7%)
Miscellaneous	4 (2.4%)	2 (1.2%)	6 (3.6%)
Trauma	19 (11.5%)	21 (12.7%)	22 (13.3%)
5. Unspecified	72 (43.6%)	69 (41.8%)	75 (45,5%)
TOTAL	165(100.0%)	165(100.0%)	165(100.0%)

Table 7. Distribution of Testicular Volume According to Cytogenetic Anomaly

Cytogentic Anomaly	Macro-orchidism (P_1)	Micro-orchidism (P_2)	Normal (P_3)
Chromosome Abnormalities			
Balanced translocations	3 (1.8%)	2 (1.2%)	1 (0.6%)
Other	1 (0.6%)	5 (3.0%)	4 (2.4%)
46,XY,5p-		1 (0.6%)	2 (1.2%)
47,XY,+inv dup(15)		2 (1.2%)	
47,XXY		1 (0.6%)	
47,XYY	1 (0.6%)		
Miscellaneous		1 (0.6%)	2 (1.2%)
TOTAL	4 (2.4%)	7 (4.2%)	5 (3.0%)
Inv (3c)	5 (3.0%)	5 (3.0%)	9 (5.4%)
Autosomal lesions	16 (9.6%)	4 (2.4%)*	15 (9.0%)

*statistically significant difference, Z-test, two-sided, $\alpha = .05$ (for actual Z-scores and confidence intervals for p_i see Table 23 and 24 respectively).

Table 8. Distribution of Testicular Volume in Non-Specific XLMR

Non-specific XLMR	Macro-orchidism (P_1)	Micro-orchidism (P_2)	Normal (P_3)
Fra(X) positive	17 (10.3%)*	2 (1.2%)	2 (1.2%)
Fra(X) negative	18 (10.9%)	16 (9.7%)	15 (9.1%)
TOTAL	35 (21.2%)*	18 (10.9%)	17 (10.3%)

*statistically significant, Z-test, two-sided, $\alpha = .05$ (for Z-scores and 95% confidence intervals for $\hat{p}_i$, see Table 23 and 24 respectively).

Table 9. Distribution of Testicular Volume According to Various Genetic Factors Related to MR

Genetic Factor	Macro-orchidism (P_1)	Micro-orchidism (P_2)	Normal (P_3)
Consanguinity	3 (1.8%)	2 (1.2%)	4 (2.4%)
Autosomal dominant			
Neurofibromatosis		1 (0.6%)	
Tuberous sclerosis	2 (1.2%)		3 (1.8%)
Cranio-facial-digital syndrome			1 (0.6%)
TOTAL	2 (1.2%)	1 (0.6%)	4 (2.4%)
Autosomal recessive			
PKU	4 (2.4%)	3 (1.8%)	1 (0.6%)
Other			1 (0.6%)
TOTAL	4 (2.4%)	3 (1.8%)	2 (1.2%)
Unknown Etiology			
Sturge-Weber syndrome			1 (0.6%)
deLange syndrome		1 (0.6%)	
TOTAL		1 (0.6%)	1 (0.6%)
Non specific familial MR	16 (9.7%)	18 (10.9%)	16 (9.7%)

(for Z-scores and 95% confidence intervals for $\hat{p}_i$, see Table 23 and 24 respectively).

Table 10. Distribution of Testicular Volume According to Environmental Factors

Environmental Cause	Macro-orchidism (P_1)	Micro-orchidism (P_2)	Normal (P_3)
Prenatal Disease			
Toxoplasmosis	1 (0.6%)		1 (0.6%)
Rubella		4 (2.4%)	
Scarlet fever			1 (0.6%)
Rheumatic fever		1 (0.6%)	
Syphilis	1 (0.6%)		
TOTAL	2 (1.2%)	5 (3.0%)	2 (1.2%)
Post-natal Disease			
Encephalitis		7 (4.2%)	5 (3.0%)
Meningitis	1 (0.6%)	7 (4.2%)	4 (2.4%)
High fever/Infection	2 (1.2%)	3 (1.8%)	
Cerebral hemorrhage		1 (0.6%)	2 (1.2%)
Brain tumour	1 (0.6%)		
TOTAL	4 (2.4%)*	18 (10.9%)	11 (6.7%)
Miscellaneous			
Status epilepticus	1 (0.6%)		
Hypothyroidism			1 (0.6%)
Cranial calcifications			1 (0.6%)
Lead poisonning			1 (0.6%)
Meningeal adhesions			1 (0.6%)
ABO incompatibility			1 (0.6%)
Psychiatric disorder	3 (1.8%)	2 (1.2%)	1 (0.6%)
TOTAL	4 (2.4%)	2 (1.2%)	6 (3.6%)
Trauma			
Birth injury	7 (4.2%)	8 (4.8%)	7 (4.2%)
Anoxia	6 (3.6%)	7 (4.2%)	8 (3.8%)
Post-natal injury	6 (3.6%)	6 (3.6%)	7 (4.2%)
TOTAL	19 (11.5%)	21 (12.7%)	22(13.3%)
Unknown	72 (43.6%)	69 (41.8%)	75(45.5%)

*statistically significant, Z-test, two sided, $\alpha = .05$ (for Z-scores and confidence intervals for $\hat{p}_i$, see Table 23 and 24 respectively).

Table 11. Distribution of Testicular Volume According to Neurological Disorders

Neurological Disorder	Macro-orchidism (p_1)	Micro-orchidism (p_2)	Normal (p_3)
Spastic/Paralytic Disorders			
Spastic paralysis	0	40 (24.2%)*	9 (5.4%)
Paralysis	1 (0.6%)	12 (7.2%)*	5 (3.0%)
Cerebral palsy	1 (0.6%)	4 (2.4%)	1 (0.6%)
Cerebral palsy with spastic quadriplegia	0	3 (1.8%)	3 (1.8%)
Undefined spasticity	5 (3.0%)	7 (4.2%)	8 (4.8%)
TOTAL	7 (4.2%)	66 (39.8%)	26(15.6%)
Epilepsy	67 (40.2%)	81 (48.6%)	71(42.6%)

*statistically significant, Z-test, two-sided, $\alpha = .05$ (for Z-scores and confidence intervals for $\hat{p}_i$, see Table 23 and 24 respectively).

Table 12. MR Diagnostic Groups in Cryptorchid Males

Diagnosis	No. Cryptorchid Males
Chromosome Abnormality	
47,XY,+ inv dup(15)	1(10%)
Other Genetic Factors	
Non-specific familial MR	2(20%)
Environmentally caused	
Pre-natal disease (rubella)	1(10%)
Post-natal disease (encephalitis)	1(10%)
Unspecified	5(50%)
TOTAL	10(100%)

Table 13. Mean Heights for Males with Non-specific XLMR

Non-specific XLMR	Height (cm)
Fra(X) positive	169.3 ± 6.5
Fra(X) negative	
+ macro-orchidism	170.4 ± 7.7
+ micro-orchidism	163.0 ± 10.2
+ normal volume	168.2 ± 8.8

Analysis of variance, $F=1.973$, $\alpha=.05$

Table 14 Testicular Volume and Age of Fra(X) Males

Resident	Testicular Volume (ml)	Age (yr)
A.Bl.	11.3	11
S.Bl.	53.2	20
D.B.	11.8	21
W.B.	44.9	34
R.W.	33.1	43
A.C.	17.1	66
W.C.	42.3	60
T.C.	63.3	65
D.D.	18.3	20
D.M.	33.8	28
A.Mt.	60.7	21
L.Mt.	95.6	30
A_2D.	55.7	58
A_1D.	80.3	57
R.Eg.	37.7	21
B.O.	6.7	23
J.Bo.	14.4	67
J.H.W.	24.4	32
M.M.	29.3	27
K.Fr.	33.0	34
R.Wk.	65.4	20
D.Bc.	65.4	20
J.K.	71.6	46
D.R.	100.0	33
H.L.	119.9	23
Average Volume ($\bar{V}$)	49.1 $\pm$ 29.5	

Table 15 Mean Percentage of Fra(X) Expression in Affected Males

Patient	Level of MR	% Fra(X)	Age(yr)	$\bar{X}$	N
L.Mt.	Profound	26	30		
M.M.	"	6	27		
R.Bc.	"	40	16		
R.F.	"	15	21		
D.R.	"	38	33	25.0±14.6	5
B.O.	Severe(20)	27	23		
J.Bo.	"(23)	11	67		
D.B.	"	10	21		
W.B.	"	24	21		
D.Br.	"	16	20		
T.C.	"(31)	30	65		
W.C.	"	56	60		
A.C.	"	20	66		
A_2.D.	"(34)	22	58		
D.M.	"	10	28		
J.H.W.	"	33	32		
R.Wo.	"(32)	30	20		
S.Bl.	"(30)	25	20		
A.Mt.	"	32	21	24.4±12.1	15
J.Dn.	"	20	30		
A_1.D.	Moderate(37)	10	57		
D.D.	" (30)	20	20		
D.Bc.	" (36)	27	20		
A.Bl.	" (36)	25	11		
H.L.	"	50	23		
K.A.	"	38	12	27.7±14.1	7
K.E.	"(40-54)	24	34		
J.K.	Mild (51)	14	46		1
C.Dn.	Not tested	22	21		
D.Dn.	"	34	26		
R.Y.	"	21	25	-	3
R.M.	"	2	25		
$\bar{X}$				25.0±12.0	31

analysis of variance; $F=-2.015$, $\alpha=.05$

$\bar{X}$ = mean % fra(X)

Table 16 Percentage of Fra(X) Expression and MR in Heterozygotes

Patient	Presence of MR	% Fra(X)	Age(yr)	$\bar{X}$	N
D.A.	Nil	53	21		
F.A.	"	12	20		
G.A.	"	[illegible]	46		
L.A.	"	30	26		
M.D.	"	0	45		
B.D.	"	2	50		
R.Bc.	"	8	45		
R.C.	"	4	75		
G.D.	"	0	66		
R.D.	"	20	22		
S.D.	"	26	22		
N.F.	"	18	24		
E.P.	"	0	58		
P.A.	"	2	30		
M.A.	"	0	50		
R.Y.	"	10	14		
M.B.	"	0	65		
B.M.	"	0	55		
P.F.	"	2	68		
B.Y.	"	4	47	10.0	20
G.B.	+	4	40		
J.Br.	+	24	14		
R.Mt.	+	8	31		
D.O.	+	16	45		
W.Wo.	+	14	42		
T.M.	+	12	22	13.0	6
TOTAL				10.6	26

$\bar{X}$ - mean % fra(X)
N - sample size

Table 17 Fra(X) Frequency (%) in Repeated Lymphocyte Cultures

Patient	Culture			
	#1	#2	#3	#4
D.Bc.	27	26	27	25
R.W.	30	16	32	24
D.R.	38	20	38	34
A.Mt.	32	32	32	-
S.Bl.	25	Refused	-	-
J.Bo.	6	11	-	-
W.B.	56	24	-	-
R.Wo.	8	34	-	-
W.C.	6	Failure	-	-
T.C.	30	24	-	-
A_1.D.	2	10	-	-
A_2.D.	2	22	-	-
D.D.	20	16	-	-
R.F.	15	12	-	-
K.Fr.	24	20	-	-
J.K.	14	10	-	-
H.L.	50	38	-	-
M.M.	6	6	-	-
B.O.	16	27	-	-
J.H.W.	33	18	-	-

paired t-test for correlation, two-sided, $t=.847$, $\alpha = .05$

Table 18 Testicular Volume and Other Chromosome Abnormalities

Patient	Chromosome Abnormality	Testicular Volume (ml)	Age (yr)
M.K.	46,XY,del(5)(p14→pter)	23.4	38
M.Bg.	46,XY,del(5)(p14→pter)	8.9	20
G.B.	46,XY,del(5)(p14→pter)	16.3	37
H.W.	47,XY,+inv dup(15)(p13→q12)	3.4	43
C.V.	47,XY,+inv dup(15)(p13→q12)	8.8	22
R.Mc.	47,XY,+inv dup(15)(p13→q12)	cryptorchid	32
B.V.	46,XY,t(5;14)(q22;q31)	normal by orchidometer	32
R.W.	46,XY,t(14;15)(q32;q13)	27.1	28
T.W.	46,XY,t(14;15)(q32;q13)	13.7	22
R.K.	45,XY,-13,-14,+t(13q14q)	27.6	34
K.S.	46,XY,t(16;22)(p12;q13)	36.7	22
J.T.	46,XY,t(7;16)(q11;p13)	infantile	21
R.M.	47,XXY	3.7	28
A.S.	47,XYY	46.6	58
K.C.	46,XY,del(10)(p13→pter)	15.6	26
T.Wb.	46,XY,der(8q+)?	18.4	24
S.Cn.	46,XY,dup(21)(q22)	infantile	30

Table 19 Frequency of 3(c) Pericentric Inversions in Fetuses Tested for Advanced Maternal Age

Year	Total # Examined	# Inv (3c)	%
1974-75	18	0	0
1976-77	71	1	1.4
1978	104	3	2.9
1979-80	137	8	5.8
1981-82	131	6	4.6
1982-83	190	5	2.6
TOTAL	651	23	3.5

Table 20. STRATIFICATION VALUES OF P_o

Event	P_o	Standard Deviation	Stratified Percentage
Chromosome Abnormalities			
Balanced translocations	.0109	.0004	1.1
46,XY,5p-	.0091	.0004	.9
47,XY,+inv dup(15)	.0082	.0009	.8
47,XXY	.0026	.0002	.3
47,XYY	.0013	.0001	.1
Other	.0065	.0003	.7
Total	.0360	.0011	3.6
Inv(3q)	.0370	.0005	3.7
Autosomal lesions	.0602	.0008	6.0
Non-specific XLMR			
Fra(x) positive	.0305	.0005	3.1
Fra(X) negative	.0937	.0007	9.4
Total	.1242	.0011	12.4
Non-specific MR	.1043	.0014	10.4
Consanguinity	.0169	.0005	1.7
Autosomal dominant	.0130	.0004	1.3
Autosomal recessive	.0167	.0005	1.7
PKU	.0130	.0004	1.3
Syndromes of unknown etiology	.0046	.0003	.5
Pre-natal Disease	.0225	.0010	2.3
Post-natal Disease	.0763	.0013	7.6
Miscellaneous	.0221	.0005	2.2
Trauma	.1217	.0012	12.2
Unspecified	.4365	.0022	43.7
Spastic/Paralytic Disorders	.2369	.0016	23.7
Epilepsy	.4430	.0016	44.3

Table 21. Clinical Summary of Fra(X) Males

PATIENT	J.B.	H.L.	B.O.	D.D.	D.M.	J.H.W.	D.Br.	R.Mo.	D.R.	W.B.	T.C.	W.C.	A.C.	K.Mo.	S.Bl.	A.Bf.	D.Bc.	R.Wh.	J.R.	R.Fg.	D.R.	A.Mt.	L.Mt.	A_1+D	A_2+D	K.F.
Age (yr)	67	25	23	20	28	32	18	43	21	34	65	60	66	27	20	11	20	20	46	21	33	21	30	57	58	34
Height (cm)	165	175	154	168	169	173	175	174	160	161.5	172	174	183	173.5	170	145	171	170.5	173	164	166	167	172	163	160	179
Birth Weight (gm)	nr	2810	nr	4037	2994	nr	nr	nr	nr	3402	4082	nr	nr	2631	4219	nr	3765	3901	nr	nr	nr	3630	3085	N	N	nr
Head Circumference (cm)	55	nr	56	57	59	57.5	56.5	60	58	57.5	54	54	nr	54	59.5	57.8	58.3	56.5	nr	nr	nr	nr	N	Large	N	
Jocular Speech	x	x	None	N	None	-	N	x	x	x	-	-	-	ltd.	ltd.	-	-	N	x	x	quibbles	x	x	x	x	x
Long face	x	x	-	x	Large head	Broad	-	-	x	-	-	-	-	x	-	x	-	-	-	x	-	x	x	x	broad large	x
High Forehead	Flat	High	-	-	x	x	-	-	-	x	-	-	-	x	x	x	-	-	-	-	x	-	-	-	-	-
Prominent Chin	x	-	x	-	-	-	-	-	-	x	square	-	x	-	-	-	-	-	-	-	x	x	x	-	-	-
High Arched Palate	-	-	x	-	x	-	-	-	x	-	-	-	-	-	-	-	-	x	-	slight cleft		-	narrow	x	x	
Large Protruding Ears	x	x	x	x	x	x	small	x	-	x	x	x	x	large top pinned to head	-	-	-	-	x	-	-	-	-	-	slight	-
Broad Nose	x	-	x	x	x	x	x	x	x	x	x	-	x	-	-	-	-	-	x	-	-	x	long thin	x	x	
Long Fingers, Hands & Feet	x	-	x	x	small	-	x	-	-	x	x	-	-	-	-	-	-	-	x	-	-	x	-	broad short	x	-
Hyperactive	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	x	-	x	-	-	-	-	-	-	-	-
Macro-orchidism	-	x	-	-	x	-	-	x	-	x	x	x	-	x	x	x	x	x	x	x	x	x	x	x	x	x
Micro-orchidism	x	-	x	-	-	-	-	-	x	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Normal testicular volume	-	-	-	x	-	x	-	-	-	-	-	-	x	-	-	-	-	-	-	-	-	-	-	-	-	-
Kyphosis	T		T		T		T*				T	dorsal														
Babinski's	none	none		N	none	N		neg	neg	neg	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
Autism	-	x	-	-	x	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Other	High diaphragm, aortic knob	-	-	-	PKU variant	-	-	-	-	-	acromegaly left sinlen crease	right hydrocele	-	-	-	-	bilateral inguinal hernia	tunnel chest	-	left inguinal hernia	gynecomastia	-	-	-	enlarged prostate	-

X = characteristic present - = characteristic absent ltd. = limited N = Normal nr = not recorded T = thoracic * = scoliosis

Table 22 Percentage of Down Syndrome in Institutions for the Mentally Retarded*

Regional Centre	Males			Females			Males & Females	
	Down syndrome	Non-Down	Total #	Down syndrome	Non-Down	Total #	Total # Down syndrome	Total # Residents
Southwestern	55	319	374	22	211	233	77	607
Huronia	82	531	613	40	229	269	122	882
Oxford	40	342	382	19	229	238	59	620
S.T.A.R.T.	4	84	88	3	44	47	7	135
Total	181	1276	1457	84	713	787	265	2244
% Down syndrome	12.4%			10.7%			11.8%	

*Numbers courtesy of Medical Records, Southwestern, Huronia, Oxford, and S.T.A.R.T. Regional Centres.

Table 23. Z-Scores for Differences between $\hat{P}_i$'s

Event	Z-Scores ($\alpha = .05$)		
	$H_o: \hat{P}_1 = \hat{P}_2$ $H_a: \hat{P}_1 \neq \hat{P}_2$ ($\alpha = .017$)	$H_o: \hat{P}_2 = \hat{P}_3$ $H_a: \hat{P}_2 \neq \hat{P}_3$ ($\alpha = .017$)	$H_o: \hat{P}_1 = \hat{P}_3$ $H_a: \hat{P}_1 \neq \hat{P}_3$ ($\alpha = .017$)
Chromosome Abnormalities			
Balanced translocations	.466	.606	1.010
Other	1.616	.337	1.399
Total	.898	.599	.461
Inv(3c)	.817	1.552	.798
Autosomal lesions	3.105*	2.635*	.544
Non-specific XLMR			
Fra(x) positive	3.600*	0	3.600*
Fra(X) negative	.364	.187	.544
Total	2.550*	.178	2.700*
Non-specific familial MR	.357	.357	0
Consanguinity	.315	.599	.272
Autosomal dominant	.171	.945	.599
Autosomal recessive	.272	.315	.599
Syndromes of unknown etiology	0	0	0
Pre-natal Disease	1.136	1.136	0
Post-natal Disease	3.030*	1.369	1.845
Miscellaneous	.808	1.426	.638
Trauma	.337	.164	.505
Unspecified	.551	.331	.220
Neurological Disorders			
Spastic paralysis	12.306*	4.793*	3.030*
Paralysis	3.175*	1.768	1.616
Cerebral palsy	1.399	1.399	0
Cerebral palsy with SQ	1.653	0	1.653
Unspecified spasticity	.577	.264	.866
Total	7.824*	4.947*	3.922*
Epilepsy	1.543	1.102	.449

$\hat{P}_1$ = % macro-orchid $\hat{P}_2$ = % micro-orchid $\hat{P}_3$ = % normal volume (from Tables 7-11)
H_o: original hypothesis, no significant difference between $\hat{P}_i$'s
H_a: alternate hypothesis, significant difference between $\hat{P}_i$'s
SQ = spastic quadriplegia
* H_o rejected, H_a accepted (see Appendix 5)

Table 24. 95% Confidence Intervals for Estimates of $\hat{P}_i$

Event	$\hat{P}_1$	$\hat{P}_2$	$\hat{P}_3$
Chromosome Abnormalities			
Balanced translocations	.018 ± .020	.012 ± .017	.030 ± .026
Other	.006 ± .0.2	.030 ± .026	.025 ± .023
Total	.024 ± .024	.042 ± .031	.030 ± .025
Inv(3c)	.030 ± .025	.030 ± .025	.054 ± .033
Autosomal lesions	.104 ± .016	.026 ± .024	.090 ± .043
Non-specific XLMR			
Fra(x) positive	.102 ± .046	.012 ± .017	.012 ± .017
Fra(X) negative	.108 ± .047	.096 ± .045	.090 ± .043
Total	.210 ± .062	.108 ± .047	.102 ± .046
Consanguinity	.018 ± .020	.012 ± .016	.024 ± .024
Autosomal dominant	.012 ± .016	.006 ± .012	.024 ± .024
Autosomal recessive	.024 ± .024	.018 ± .020	.012 ± .016
Syndromes of unknown etiology	0	.006 ± .012	.006 ± .012
Non-specific familial MR	.096 ± .045	.108 ± .047	.096 ± .045
Pre-natal Disease	.012 ± .017	.030 ± .026	.012 ± .017
Post-natal Disease	.024 ± .024	.108 ± .047	.066 ± .038
Miscellaneous	.024 ± .024	.012 ± .017	.036 ± .028
Trauma	.114 ± .049	.126 ± .051	.132 ± .052
Unspecified	.436 ± .076	.418 ± .075	.455 ± .076
Spastic/Paralytic Disorders			
Spastic paralysis	0	.242 ± .065	.054 ± .035
Paralysis	.006 ± .012	.072 ± .039	.030 ± .026
Cerebral palsy	.006 ± .012	.024 ± .024	.006 ± .012
Cerebral Palsy with SQ	0	.018 ± .020	.018 ± .020
Unspecified spasticity	.030 ± .026	.042 ± .031	.048 ± .033
Total	.042 ± .031	.398 ± .075	.174 ± .057
Epilepsy	.402 ± .074	.486 ± .076	.426 ± .074

$\hat{P}_1$ = macro-orchidism $\hat{P}_2$ = micro-orchidism $\hat{P}_3$ = normal testicular volume
SQ = spastic quadriplegia

Table 25 Testicular Volume in Repeated Measurements

Patient	Testicular Measurements					
	l_1 (cm)	w_1 (cm)	V_1 (ml)	l_2 (cm)	w_2 (cm)	V_2 (ml)
A.C.	3.9	2.9	17.1	4.2	3.1	20.5
D.B.	4.3	2.3	11.8	4.1	1.9	7.7
D.D.	6.1	2.4	18.3	5.9	2.3	16.2
B.O.	3.2	2.0	6.7	3.7	1.7	5.6
J.H.W.	4.2	3.3	24.4	4.2	2.8	17.1

APPENDICES

1. Clinical Summary of Patients with Other Chromosome Abnormalities

(a) 47,XXY (Figure 32)

R.M. was a white male, born August 28, 1947. The birth weight was 2858 gm after a full term, normal pregnancy. The mother was 36 and the father 38 at the time of his birth. The patient was the last of four pregnancies (Figure 33), the second resulting in a miscarriage. There was no family history of congenital abnormalities or MR.

Clinical Features: The patient, aged 36, had a height of 154.5 cm and a head circumference of 53.4 cm. He had a slight speech defect, myopia, irregularly arranged teeth and toe nails which were thickened and deformed. The testes were small and soft.

(b) 47,XYY (Figure 34)

A.S. was a white male born February 24, 1925. The pregnancy was full term but the birth weight was not recorded. Both parents were between 26-30 years of age. The patient was the fifth of nine children (Figure 35). There was no family history of congenital abnormalities but the father was reported to be mentally deficient.

Clinical Features: The patient, aged 58 years, had a height of 184 cm. The head was large with a prominent forehead and bulbous nose. The palate was slightly high. The digits were short and broad and there was a slight speech impediment. The testes were macro-orchid.

(c) 46,XY,del(5)(p14-pter)

Three patients were found with the cri-du-chat syndrome (Figures 36, 38, 40).

(i) M.K. was a white male born June 14, 1945. The birth weight was 3674 gm after a full term, normal pregnancy. The mother was 24 and the father 30 at the time of the birth. M.K. was the fifth of six children (Figure 37). There was no family history of congenital abnormalities. A great grand-uncle was reported to be mentally retarded.

Clinical Features: The patient, aged 38, had a height of 165 cm and a head circumference of 53 cm. He was generally small, with a cleft palate, a high arch, microcephaly, and a receeding chin. The patient was aphasic. There was a systolic murmur over the aortic valve. The left testicle was of normal volume and the right was cryptorchid.

(ii) M.B. was a white male, born August 3, 1963. The birth weight was 3221 gm after a premature delivery. The mother was 31 and the father 33 at the time of birth. M.B. was the third of four children (Figure 39). There was no family history of congenital abnormalities or MR.

Clinical Features: The patient, aged 20, had a height of 169 cm and a head circumference of 56 cm. He exhibited hypertelorism, a thick nose and a small upper lip. The ears were pinned back. Speech was indistinct. He had a congenital kidney abnormality as well as cystic fibrosis. There was equinovarus of the right foot. The testes were micro-orchid.

He had thoraco-lumbar scoliosis and retinal pigmentation.

(iii) G.B. was a white male born October 21, 1946. The birth weight was 2722 gm after a full term, normal pregnancy. The mother was 23 and the father 22 at the time of birth. G.B. was fourth of seven children (Figure 41). The mother had three miscarriages. There was no family history of congenital abnormalities or mental retardation.

Clinical Features: The patient, aged 37 years, had a height of 155 cm and a head circumference of 50.7 cm. His speech was impaired and he has external stabismus of his right eye. The testes were of normal volume.

(d) 45,XY,-13,-14,+t(13q14q)(Figure 42)

R.K. was a white male born March 16, 1949. The pregnancy was full term and the birth weight was reportedly low but was not recorded. His mother, 4 siblings and 2 nephews were all found to carry the same translocation (Figure 43). None were mentally retarded. His mother (II-43) had had six spontaneous abortions; his sister (III-10) had had two miscarriages and another sister (III-14) had had seven miscarriages. A paternal female cousin (II-12) was also mentally retarded. She was not available for chromosome studies.

Clinical Features: The patient, aged 34 years, had a height of 161 cm. There was general growth failure. He had a prominent forehead and micrognathia. His digits were long and thin. Dental abnormalities were present. His trunk was very much shortened and there was a thoraco-lumbar

kyphoscoliosis. Testes were macro-orchid.

(e) 46,XY,t(14;15)(q32;q13) (Figure 44)

Two brothers were found to carry this translocation (Figure 45).

(i) R.W. was a white male born July 5, 1955. The birth weight was 3765 gm after a full term, normal pregnancy. The mother was 21 and the father 29 at the time of the birth. R.W. was the second of four children. The patient's father had the same abnormal chromosome constitution as the proband. He was considered "slow" at school and presented facial features similar to those of the propositus. Two of the mother's first cousins (once removed) had cleft palates.

Clinical Features: The patient, aged 28, had a height of 178 cm and a head circumference of 55.7 cm. The ears were large and flabby with little cartilaginous support. The eybrows almost met in the midline. The palate was high and narrow and had been cleft. The eyes were large with much of the sclera visible below the iris. Bone age was retarded. Testes were macro-orchid. Speech was impaired and very nasal.

(ii) T.W., brother of R.W., was also a white male born February 15, 1961. The birth weight was not recorded. The mother was 27 and the father 35 at the time of birth. He was the fourth of four children.

Clinical Features: The patient, aged 22, had a height of 159 cm with a head circumference of 55 cm. He had large

ears, prominent sclera visible below the iris and a high, narrow palate. The palate had also been cleft. He ~~had~~ had bilateral inguinal hernias. His speech was slurred. The testes were micro-orchid. The left testis had originally been cryptorchid.

(f) 46,XY,t(5;14)(q22;q31) (Figure 47)

B.Vs. was a white male born February 27, 1963. The birth weight was 2359 gm. Delivery was premature, at 35 weeks but uncomplicated. The mother was 31 and the father 32 years at the time of birth. B.Vs. was the first born of fraternal twins. His twin brother weighed 2132 gm at birth. His father had a retarded sister (Figure 48).

Clinical Features: The patient, aged 20 years, had a height of 176 cm and a head circumference of 59.3 cm. There was no speech. He had eversion of the left eye but otherwise his features were unremarkable. He was aggressive and psychotic. The testes were of normal volume (measured by orchidometer).

(g) 46,XY,t(16;22)(p12;q13) (Figure 49)

K.S. was a white male, born October 12, 1961. The birth weight was 3674 gm after a normal pregnancy. The mother was 32 and the father 34 at the time of birth. The patient was the youngest of 7 children. There was no family history of congenital abnormalities or mental retardation (Figure 50).

Clinical Features: The patient, aged 22, had a height of 177 cm and a head circumference of 59 cm. His features

were unremarkable and he had no speech. The Babinski was downgoing and other reflexes were also normal. He had lowered foot arches and there was webbing of the 2nd and 3rd toes. The testes were macro-orchid.

(h) 46,XY,t(7;16)(q11;p13) (Figure 51)

J.T. was a white male born May 7, 1962. There was no information concerning his family as he was adopted at a very early age.

Clinical Features: The patient, aged 21, had a height of 168 cms and a head circumference of 54 cm. He had small ears, a large nose, thick lips and a small high palate. There was lumbar lordosis and he walks with his feet inverted. He had an inguinal hernia. He had radio-ulnar synostosis and epilepsy.

(i) 46,XY,der(8q+)? (Figure 52)

T.W. was a white male, born July 17, 1959. The birth weight was 3674 gm after a full term, normal and breech delivered pregnancy. The mother was 23 and the father 27 at the time of birth. T.W. was the third of four children (Figure 53). The mother had one miscarriage at three months.

Clinical Features: The patient, aged 24, had a height of 159 cm and head circumference was listed as normal but was not recorded. His head was asymmetrical with a flattened left occiput. He had micrognathia and had had a tumor in his right sternomastoid. His hearing was normal despite very tiny ears and ear canals. His eyes had a marked light brown

pigmentation lattice work on the outer side of the iris. The palate was highly arched, especially on the right side. There were multiple skeletal anomalies including a deformed right cranium, bell shaped chest, a lateral bowing of the left elbow, and a deformed right leg. Tooth development was irregular. There was erroneous endocrine function. The reflexes were hyperactive ankle clonus and both plantars are upgoing. There was a dorsal scoliosis. He had a slight lordosis with a wide ape-like stance and gait. There was bilateral pes planis. The fingers were extremely long with tapering digits. He had large amounts of body hair along the spine. The right testis was micro-orchid and the left was originally cryptorchid. T.W. was extremely strong and unco-operative.

(j) 46,XY,dup(21)(q22)(Figures 54, 55)

S.Cn. was a white male, born September 8, 1957. The birth weight was 2812 gm after a full term, normal pregnancy. The mother was 22 and the father 30 at the time of the birth. The patient was the third of 4 siblings (Figure 56). There was a mentally retarded paternal uncle and three of the father's siblings died at birth. S.Cn.'s mother was found to have a mosaic karyotype: 46,XX/47,XXX/48,XXXX (courtesy of Dr. Markovic, Surry Place, Toronto, Ontario).

Clinical Features: The patient, aged 30, had a height of 164 cm with a slightly enlarged head circumference but the actual measurement was not recorded. He was generally small,

with small protruding ears, downward sloped eyes, epicanthal folds, small chin and a roundish face. His facial appearance was similar but not typically representive of Down syndrome The palate was very high and narrow with a medium cleft. He had clinodactyly of phalynx 5. He was echolalic but the speech was indistinct. He had a mild deformity of the sternum with a slight dorsal kyphosis and an awkward gait. There was no simian crease and no Babinski. The penis was small and the testes micro-orchid. SOD levels (660ng/mg hemoglobin) were found to be in the normal range (600-800ng/mg hemoglobin).

(k) 46,XY,del(10)(p13→pter) (Figure 57)

K.C. was a white male born September 26, 1957. The birth weight was 2631 gm after a 3 week premature pregnancy with a normal delivery. The mother was 16 and the father 25 at the time of birth. K.C. was the first of two children (Figure 58). Both parents were of sub-normal intelligence. Two of the mother's siblings died as infants.

Clinical Features: The patient, aged 26, had a height of 157 cm and a head circumference of 50 cm. He was generally small. He had a long face, with a square chin, small protruding, low-set ears, microcephaly, a thick lower lip, high narrow palate and a protruding tongue. He had large hands and feet with over-riding index fingers. The epiphysis of the long and short bones were hypertrophic. The deep tendons were hyper-reflexive and the Babinski was upgoing on the right side. Shoulders and arms exhibited

jerky movements. He had constant right esotropia. He had had a small right inguinal hernia. His testes were of normal volume.

(1) 47,XY,+inv dup(15)(p13-q12)

Three residents were found to have a 47,XY karyotype with an extra bisatellited chromosome marker (Figures 59-61). These markers were each shown to possess two functional centromeres and nucleolus organizing regions (Fig. 62). The markers were conclusively shown to be inversion duplications of the proximal portion of chromosome 15 through methyl green/DAPI staining (Figure 63).

(i) H.W. was a white male born April 17, 1940. The birth weight was normal (but not recorded) after a full term normal pregnancy. The mother was 28 and the father 32 at the time of his birth. The patient was the last born of fraternal twins. He and his twin sister were the fourth of four pregnancies (Figure 65). There was no family history of congenital abnormalities or mental retardation.

Clinical Features: The patient, aged 43 years, had a height of 154.5 cm and a normal head circumference (but not recorded). He had a broad face with a high forehead, small chin, thick bulbous nose, large protruding ears and a thick protruding lower lip. He was echolalic. He had had a blood tumour on his spine. There was no Babinski's and other tendon reflexes were brisk. He walked with his knees permanently flexed. The left testis was undescended and the

right was micro-orchid.

(ii) R.McF. was a white male, born May 8, 1951. The birth weight was 3538 gm after a full term, normal pregnancy. The mother was 37 and the father 38 at the time of birth. The patient was the second of two children (Figure 64). There was no family history of congenital abnormalities or mental retardation.

Clinical Features: The patient, aged 32 years, had a height of 160 cm and a head circumference of 60.8 cm. He had an acromegaloid face with a thick bulbous nose and thick protruding lips. He had a bony hypertrophy of the temporal aspect of the superciliary ridges. He had flat feet with varus. He was obese and exhibited gynecomastia. He also had esotropia. He was epileptic and a spastic quadraplegic. The Babinski was downgoing and other reflexes were brisk. He had had an enlarged thymus in infancy. His lymph nodes were enlarged. The testes were crytorchid.

(iii) C.V. was a white male born May 13, 1961. The birth weight was 3810 gm after a full term, normal pregnancy. The mother was 41 at the time of the birth. There was no information about the father. The patient was the only child of this union (Figure 66). There was no family history of congenital abnormalities or mental retardation.

Clinical Features: The patient, aged 22 years, had a height of 167 cm, and a head circumference of 54.5 cm. He had a long rectangular face with a thick, bulbous nose and a

short frenulum linguae. He had had a high pitched voice when young. He had a severe speech impairment. The patient had mild quadriplegia with choreoathetosis. There was a mild kyphosis and he walked with a stooped gait. The testes were micro-orchid and were originally undescended.

2. Statistical Analyses

a) Z-test for the difference between two probabilities

Statistical differences between the success probabilities ($\hat{p}_i$) in each volumetric group were determined by the Z-test for the difference between two success probabilities. The Z-scores (Table 23) were calculated according to the following equations:

1) $$p = \frac{B_1 + B_2}{m+n}$$

2) $$\hat{SD}(\hat{p}_1-\hat{p}_2) = \sqrt{\frac{p(1-p)}{m} + \frac{p(1-p)}{n}}$$

3) $$Z = \frac{\hat{p}_1-\hat{p}_2}{\hat{SD}(\hat{p}_1-\hat{p}_2)}$$

where p = common success rate in two samples

B_1 = number of successes in sample 1

B_2 = number of successes in sample 2

m = sample size of sample 1

n = sample size of sample 2

$\hat{p}_1$ = success probability of sample 1

$\hat{p}_2$ = success probability of sample 2

b) Confidence Intervals (Table 20)

The 95% confidence intervals for the estimate of the success probabilities ($\hat{p}_i$) given in Table 24 were calculated according to the following formula:

$$\hat{\hat{SD}}_{\hat{p}_i} = \sqrt{\frac{\hat{p}_i(1-\hat{p}_i)}{N}}$$

where $\hat{p}_i$ = success probability

$\hat{\hat{SD}}$ = standard deviation

N = sample size

The 95% confidence interval was defined as $\hat{p}_i \pm (1.96)\ \hat{\hat{SD}}_{\hat{p}_i}$ ($Z_{.05} = 1.96$).

c) Stratum Means and Standard Deviations

In order to account for the different proportions of institutionalized males in the three volumetric groups, the overall frequencies (P_o) of the various disorders in the initial study population had to be estimated in terms of stratum means and variances. The following formulae were employed:

1) $$P_{overall} = \sum_{i=1}^{3} \left(\frac{N_i}{N}\right) \hat{p}_i$$

2) $$\hat{\hat{SD}}\ (P_o) = \sum_{i=1}^{3} \sqrt{\left(\frac{N_i}{N}\right)^2 \frac{Var(\hat{p}_i)}{N_i}}$$

3) $$Var\ (\hat{p}_i) = \frac{\hat{p}_i(1-\hat{p}_i)}{N_i}$$

where P_o = success probability in the total population

$\hat{P}_i$ = success probability in each volumetric group

N_i = size of each volumetric group

N = total adult study population

$\hat{SD}$ = standard deviation

3. Distribution of Fra(X) Negative Non-specific XLMR (excluding singly affected sibships)

	Macro-orchidism (P_1)	Micro-orchidism (P_2)	Normal (P_3)
Fra(X) Negative Non-specific XLMR	12 (7.3%)	14 (8.5%)	7 (4.2%)

Event	Z-scores		
	Ho: $\hat{p}_1=\hat{p}_2$ Ha: $\hat{p}_1\neq\hat{p}_2$ (α=.017)	Ho: $\hat{p}_2=\hat{p}_3$ Ha: $\hat{p}_2\neq\hat{p}_3$ (α=.017)	Ho: $\hat{p}_1=\hat{p}_3$ Ha: $\hat{p}_1\neq\hat{p}_3$ (α=.017)
Fra(X) Negative Non-specific XLMR	.404	1.571	1.166

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